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ELAPHOSTRONGYLUS ODOCOILEI HOBMAIER AND HOBMAIER, 1934 IN THE CRANIAL CASE OF ODOCOILEUS VIRGINIANUS BOREALIS MILLER[1]

BY ROY C. ANDERSON

Abstract

Metastrongyles, referred to *Elaphostrongylus odocoilei* Hobmaier and Hobmaier, 1934, were found in the subarachnoid space of *Odocoileus virginianus borealis* Miller from Algonquin Park, Ontario. These nematodes are described in detail and compared with the original description of *E. odocoilei*.

Introduction

On May 27, 1955, Dr. D. M. Davies of McMaster University and Mr. G. F. Bennett of the Ontario Research Foundation collected a number of metastrongyles from the cranial case of a deer from Algonquin Park, Ontario. Subsequently, 30 other deer, all from Algonquin Park, were examined during the summer of 1955 and similar metastrongyles were found in 10 of them. The 11 deer, infected with these nematodes, were from two to eight years of age as determined by Mr. R. Hepburn and Mr. R. O. Standfield of the Ontario Department of Lands and Forests. Of the 20 deer in which metastrongyles were not found, eight were fawns and the other 12 were from one to five years of age.

The author is of the opinion that these nematodes belong to *Elaphostrongylus odocoilei* Hobmaier and Hobmaier, 1934 which was collected originally from the Columbian black-tailed deer (*Odocoileus columbianus*) of California (5). Hobmaier and Hobmaier collected their specimens from the connective tissues surrounding the veins beneath the spine as well as in connective tissues surrounding the adjacent branches of these veins. The description of *E. odocoilei* is, in a few particulars, at variance with the present author's observations on the metastrongyles from deer in Ontario. In each instance, however, these differences can be safely interpreted as due either to natural variation within *E. odocoilei* or to oversight on the part of the original discoverers of the species. Nematodes which occur in the central nervous system, or associated with the meninges of their hosts, are assuming increasing

[1]*Manuscript received February 20, 1956.*
Contribution from the Department of Parasitology, Ontario Research Foundation, Toronto, Ontario.
The author is grateful to Dr. H. B. Speakman, Director, for his advice and encouragement.

[The April number of this Journal (Can. J. Zoology, 34: 79-165. 1956) was issued April 5, 1956.]

importance. It is worthwhile therefore, to describe in detail the specimens from deer in Ontario to add to the somewhat cursory description of *E. odocoilei* given by Hobmaier and Hobmaier.

The following description of the male is based on six complete specimens in addition to several fragments. The description of the female is based on nine complete female specimens in addition to numerous fragments. The dimensions given in the following description are based wholly on complete specimens. All the specimens studied are mature as determined by the presence of eggs in the female and sperm in the male.

Elaphostrongylus odocoilei Hobmaier and Hobmaier, 1934

(Figs. 1 – 12)

Description

Long, slender nematodes with thin, delicate cuticle. Teguminal sheath absent. Oral opening circular, bordered by a delicate, transparent, cuticular ring, leading into a weakly cuticularized, cone-shaped buccal capsule that is surrounded by esophageal tissue. Esophagus broad, muscular, and club-shaped. Excretory pore prominent, leading into elongated terminal duct lined with cuticle. Terminal duct leading into short, ill-defined excretory sinus, the latter dividing posteriorly into two greatly elongated, faintly granular, excretory gland cells each of which contains a large nucleus towards its distal end. Posterior end of excretory gland cells rounded. Six perityls present of equal size, each bearing a papilla (internal circle). Four submedian groups of two papillae present; each group bordered by slightly raised cuticular ridge. Ventrolateral papillae lateral to pore-like amphids, exceedingly small, and difficult to locate.

Male

Length 47 (39 – 54) mm.* Maximum width 0.19 (0.18 – 0.21) mm. Esophagus 0.71 (0.58 – 0.77) mm. in length; maximum width 0.085 (0.076 – 0.096) mm. Nerve ring 0.13 (0.11 – 0.15) mm. from anterior end. Excretory pore 0.13 (0.10 – 0.14) mm. from anterior end. Terminal excretory duct about 0.054 mm. in length. Length of terminal excretory duct and longest excretory gland cell 3.2 (1.7 – 4.5) mm. Spicules 0.21 (0.18 – 0.24) mm. in length. Gubernaculum consisting of crura and corpus. Crura heavily sclerotized, 0.022 (0.020 – 0.026) mm. in length and about 0.011 mm. in maximum width. Ventrolateral surfaces of crura with about four to six scallops. Corpus weakly sclerotized, practically indistinguishable in ventral view but discernible in lateral view and in cross section (Fig. 12 *a* – *m*). Corpus consisting of a continuous thickening of the ventral wall of the spicular pouch; lateral parts of corpus thickest. Corpus, 0.085 (0.072 – 0.10) mm. in length, broadest in its middle parts but narrow distally. Bursa well-developed about 0.10 – 0.11 mm. in length and about 0.10 – 0.12 mm.

* *The average is followed by the range.*

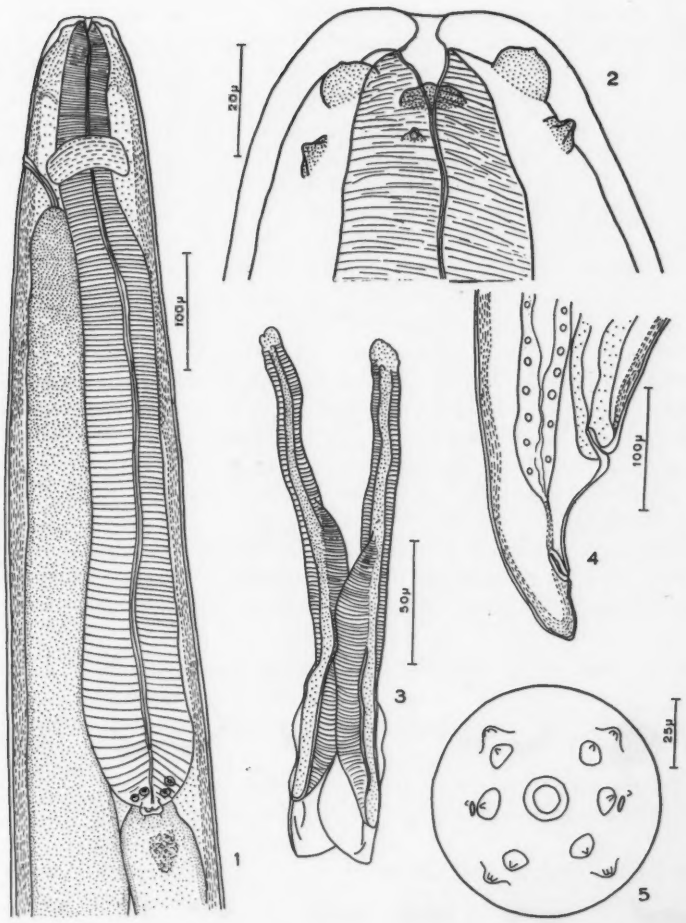


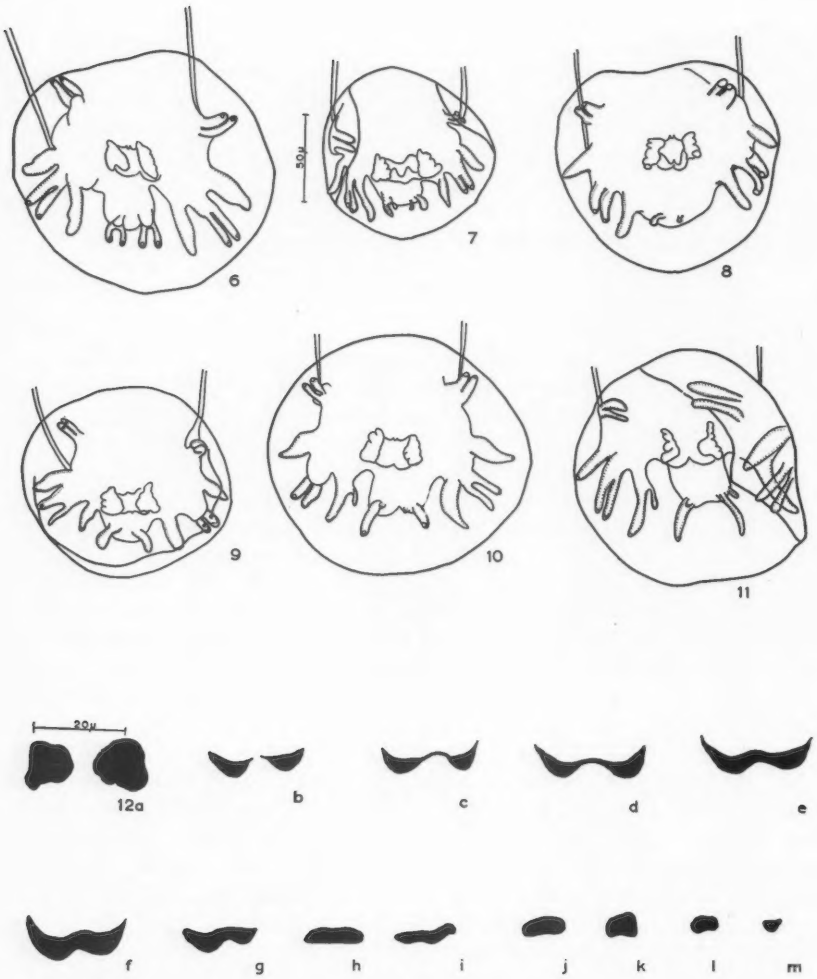
FIG. 1. Anterior end male, lateral view, showing nerve ring, esophagus, excretory pore, terminal excretory duct, and one excretory gland cell.

FIG. 2. Anterior end male, lateral view.

FIG. 3. Spicules, ventral view.

FIG. 4. Caudal end female, lateral view, showing anus, rectum, intestine, vulva, and posterior part of ovejector.

FIG. 5. Anterior end female, *en face* view.



FIGS. 6 – 11. Caudal end males, ventral views, showing crura and bursal characteristics (see text).

FIG. 12, *a* – *m*. Serial cross sections (6 μ) through gubernaculum. *a* = crura; *b* – *m* = corpus. One section through crura missing.

in width. Rays elongated and well-developed consisting of partially united ventroventrals and lateroventrals, separate externolaterals, partially united mediolaterals and posterolaterals, and separate externodorsals. Dorsal ray mound-like, variable in structure, separated into two branches each of which may or may not be further subdivided into two other branches (see Figs. 6 – 11 for variability in this structure. Specimens illustrated as follows: Fig. 6 = male No. 7, 7 = No. 5, 8 = No. 8, 9 = No. 4, 10 = No. 2, 11 = No. 3. Male specimen No. 6 is similar to specimen No. 5 (Fig. 7) and male specimen No. 1 is more or less similar to specimen No. 3 (Fig. 11). The bursa in two fragments is like that illustrated in Fig. 6).

Female

Length 62 (51 – 91) mm. Maximum width 0.20 (0.18 – 0.22) mm. Esophagus 0.75 (0.62 – 0.96) mm. in length; maximum width 0.081 (0.072 – 0.094) mm. Nerve ring 0.12 (0.099 – 0.18) mm. from anterior end. Excretory pore 0.13 (0.092 – 0.19) mm. from anterior end. Terminal excretory duct about 0.051 mm. in length. Length of terminal excretory duct and longest excretory gland cell 3.2 (2.3 – 4.7) mm. Vulva 0.15 (0.11 – 0.21) mm. from caudal extremity. Vulva leading into vagina and ovejector which have a combined length of 1.6 (1.4 – 1.8) mm. Unsegmented eggs in ovejector and uteri circular with smooth transparent shells, about 20 – 26 μ in diameter. Anus 0.048 (0.031 – 0.065) mm. from caudal extremity. Cuticle of caudal extremity loose in appearance and generally trilobed (these caudal protuberances, which are variable in shape and size, appear to consist of cuticle only and they are not interpreted here as sensory papillae).

Host: *Odocoileus virginianus borealis* Miller (type host – *O. columbianus*).

Locality: Algonquin Park, Ontario, Canada (type locality – California, U.S.A.).

Location: Between pia and dura mater of the cranial case; usually free in subarachnoid space but rarely extremities embedded in tissues of meninges.

Specimens: Males Nos. 1, 2, 3 and Female Nos. 1, 2, 3 lodged in the Helminthological Collection of the United States National Museum. Remainder of specimens in the Department of Parasitology, Ontario Research Foundation, Toronto 5, Ontario.

Discussion

The male specimen of *Elaphostrongylus odocoilei*, described by Hobmaier and Hobmaier (5), was only 35 mm. in length, 4 mm. less than the length of the smallest male metastrongyle in our collection. Similarily all the measurements given by the Hobmaiers are slightly less than those found in our specimens. Thus they give the maximum width as 0.15 mm. whereas

we obtained a range of 0.18 – 0.21 mm. They state that the spicules are "144 μ" in length whereas we found a range of 0.18 – 0.24 mm. Rather than thinking that the worms from deer in Ontario are a distinct species, the author is of the opinion that the Hobmaiers happened to choose a small male specimen to describe.

The Hobmaiers note that the entrance to the cloaca is ". . . guarded by two chitinous formations, each 20 μ long and 10 μ wide. The wall of the ductus genitalis nearby is strengthened by 2 small chitinous ribbons which are light brown and 67 μ long." It is clear from their comments as well as their illustration that their "chitinous formations" refer to the crura. Their description of the crura agrees with the present observations on this structure in the specimens from Ontario deer (Figs. 6 – 11). The author is of the opinion, moreover, that the two small "chitinous ribbons", mentioned by the Hobmaiers, refer to the thickened ventrolateral borders of the corpus. The distal part of the corpus was not observed by the author in dorsal or ventral view in specimens cleared in glycerin. The thickened ventrolateral borders of the corpus were observed, however, in both dorsal and ventral views of a specimen cleared in cedarwood oil. These borders of the corpus gave the impression of two "chitinous ribbons" as described by the Hobmaiers. Actually, however, the corpus is a solid structure although this is difficult to determine in unsectioned material (Fig. 12, *a* – *m*). Dougherty (4) gave an excellent illustration of the lateral view of the male of *E. odocoilei* which shows the appearance of the gubernaculum in side view; the present observations on the specimens described herein agrees with this illustration.

The dorsal ray is evidently a highly variable structure. The dorsal rays of some specimens from Ontario deer (e.g. Fig. 6) agree with the description of this structure given by the Hobmaiers. In other specimens the dorsal rays are quite different (Figs. 7 – 11). There is, at present, no reason to believe that our collection consists of more than one species for the worms are, apart from the dorsal ray, similar morphologically.

The Hobmaiers state that the ovejector and vagina are 1 mm. in length whereas we have found it to be 1.4 – 1.8 mm. The length of this structure is possibly variable and little importance can be attached to this minor difference. The brief description of the female of *E. odocoilei* given by Hobmaier and Hobmaier agrees in other respects with our observations on the specimens from Ontario deer.

Burg, Baudet, and Verwey (1) reported fatal intracranial haemorrhage in *Cervus elaphus* from Europe that was apparently associated with mature specimens of a metastrongyle in the subarachnoid space. They suggested that their specimens belong to the genus *Elaphostrongylus*. Cameron (2) described *E. cervi* from *Cervus elaphus* in Scotland and it is conceivable that this is the species reported by Burg *et al.* Recently De Guisti (3) reported that 46% of 79 deer heads, examined by him in Michigan, contained immature metastrongyles on the inner surfaces of the meninges; some were apparently projecting into the nervous tissue of the brain. These worms, according to De Guisti, caused inflammation and bleeding in the cranial case.

The presence of these metastrongyles associated with the central nervous system is a problem of considerable interest in view of the findings of Mackerras and Sandars (6) that the larvae of *Angiostrongylus cantonensis* (Chen, 1935) congregated in the central nervous system (especially the anterior portion of the cerebrum) after they were ingested. The third and fourth molts of the larvae of *A. cantonensis* took place in the nervous tissue and the adult worms emerged onto the surface of the brain about two weeks later. The worms spent two weeks in the subarachnoid space and from the 28th to the 31st day they migrated to the lungs via the venous system. The observations of Mackerras and Sandars raise the question as to whether larvae of *Elaphostrongylus* may not also spend some time in the central nervous system before taking up their final location in the connective tissues surrounding the veins below the spine. It is conceivable that immature metastrongyles found in the subarachnoid space of deer (3) may be worms which have just left the nervous tissue and are preparing to migrate to the connective tissues beneath the spine; the movements of these worms while leaving the brain tissue could be the cause of the haemorrhages that have been associated with these worms (1). Mature worms found in the subarachnoid space (1) may be worms which failed to leave the subarachnoid space and matured there. These latter worms may be able to continue the life cycle of the parasite provided that the eggs can reach the circulatory system and be carried to the lungs as has been shown to occur in worms situated around the veins beneath the spine (5).

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THE PAROTOID GLAND OF THE TOAD, BUFO AMERICANUS[1]

By David J. McCallion

Abstract

A histological study of the poison glands of the toad demonstrated that one of the components of their secretion is adrenalin or adrenalin precursor substances. The poison glands are arranged in aggregates of two or three making up the warts on the skin. Larger aggregates of poison glands on the head are the parotoid glands. Each poison gland is a large vesicle, deep in the corium of the skin, and opening through a pore in the skin by way of a conical duct. The glandular epithelium of the poison gland is a flat acellular layer of cytoplasm containing a large number of flattened nuclei. Forcibly discharged glands disintegrate, are resorbed, and are replaced by new glands regenerated from the Malpighian layer of the epidermis.

Introduction

The parotoid or parotid glands of the toad lie, one on each side, at the back of the head, just behind the eye and the ear. Their characteristics are diagnostic in the identification of species in toads. Two crystalline pharmacological agents were obtained from the toad, *Bufo agua*, by Abel and Macht (1). Bristol and Bartelmez (2) gave a brief description of the poison glands of this species of toad. Earlier, Calmels (3) had published a lengthy description of the poison glands of an unnamed species. The present paper describes the results of a histological examination of the parotoid gland of the common North American toad, *Bufo americanus*.

Materials and Methods

The toads used in this study were mature adults of both sexes collected in the environs of Toronto. They were maintained in the laboratory in a terrarium and were healthy and well fed.

The parotoid glands were excised from the toads and placed in fixatives appropriate to the following techniques. In all cases the tissues were dehydrated through dioxane and embedded in paraffin. Pieces of skin containing warts were similarly studied.

(1) Fixed in Bouin solution and subsequently stained in hematoxylin and eosin.

(2) Fixed in chilled picroalcohol – formalin and subsequently treated with periodic acid – Schiff reagent.

[1]*Manuscript received February 13, 1956.*
Contribution from the Department of Zoology, University of Toronto, Toronto, Ontario.

Fig. 1. A section through the parotoid gland of the toad showing the duct of one poison gland and, to the right, a mucous gland. H. & E. × 210.

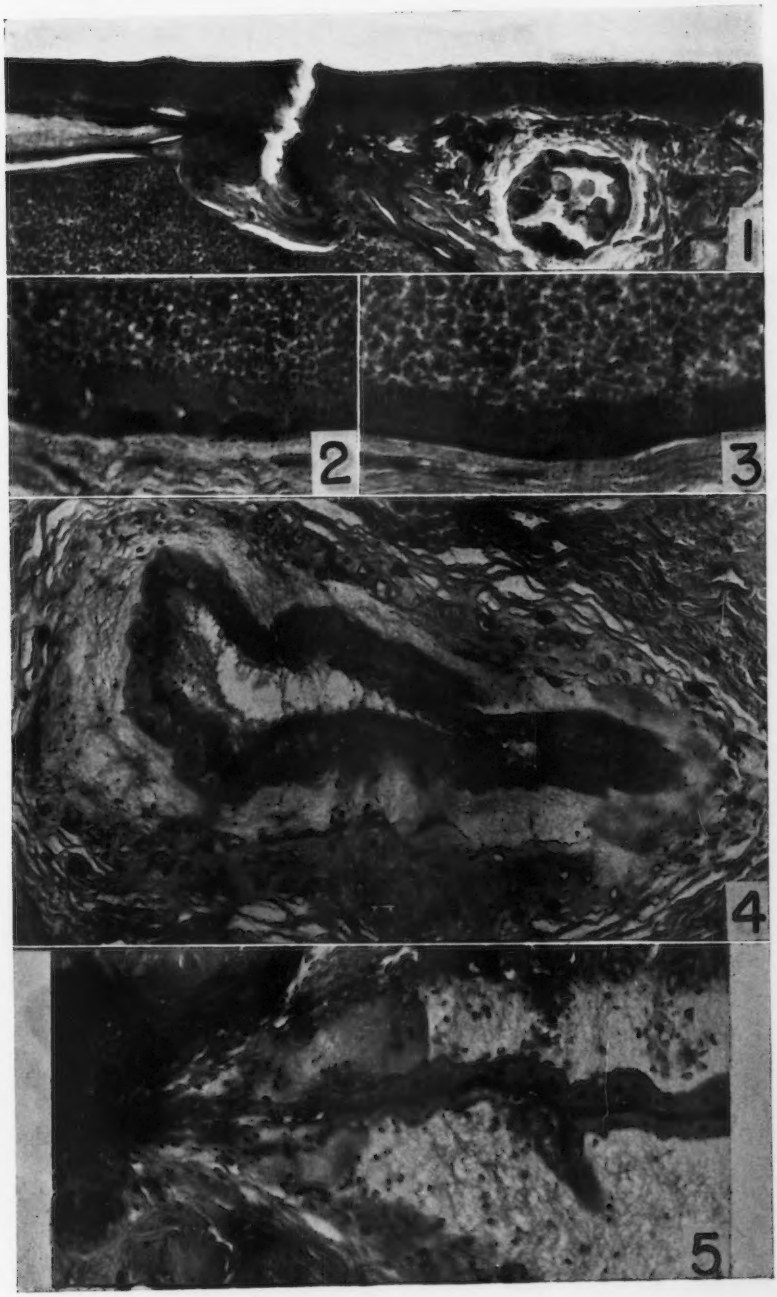
Fig. 2. A section of the wall of a single poison gland showing secretory droplets, acellular cytoplasm and nuclei, basement membrane, smooth muscle cells, and connective tissue. Schmorl stain. × 600.

Fig. 3. Same as Fig. 2. H. & E. × 600.

Fig. 4. A collapsed poison gland four and one-half hours after discharging. H. & E. × 200.

Fig. 5. Part of a collapsed poison gland 18 hr. after discharging. H. & E. × 200.

PLATE I



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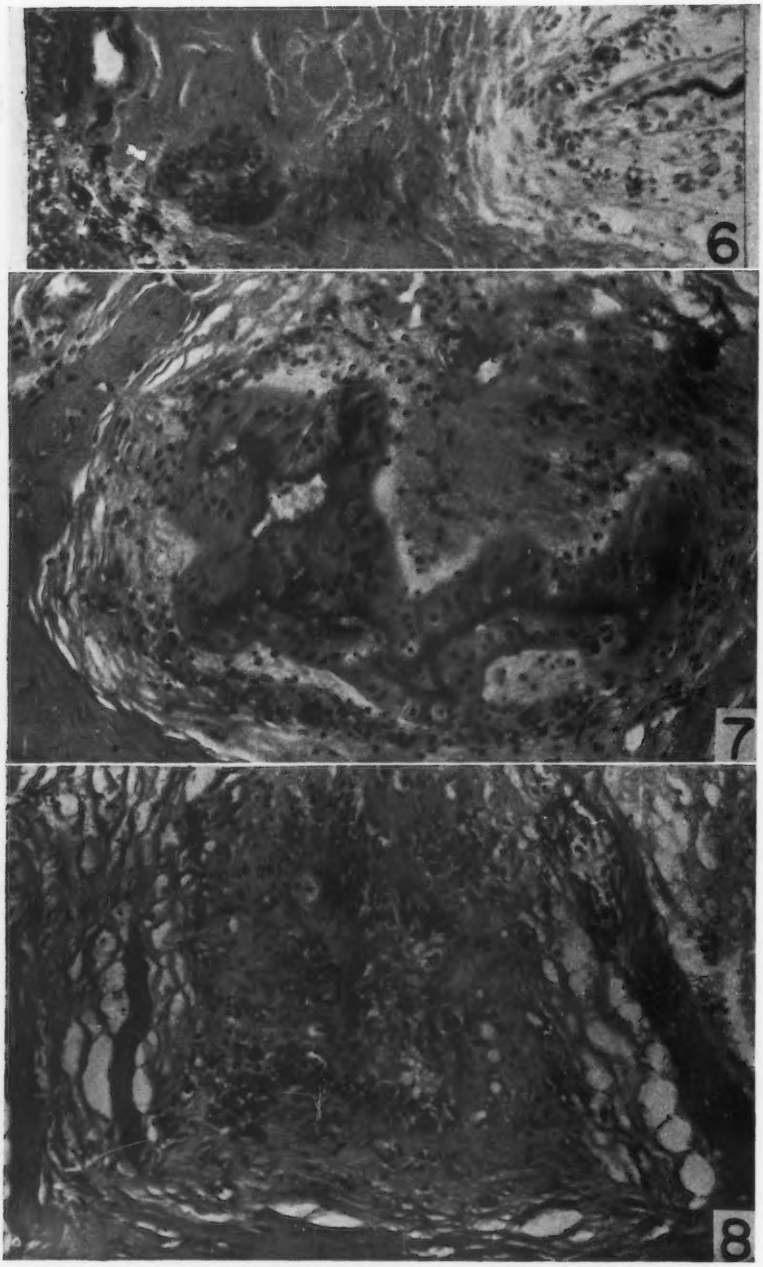

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(3) Fixed in cobalt nitrate – formalin, impregnated with silver nitrate at room temperature, and reduced in Cajal's hydroquinone mixture.
(4) Fixed in potassium bichromate – formalin (Orth) and subsequently stained in 1% aniline blue and counterstained in 1% safranin O (Schmorl).
(5) Following Bouin fixation, sections were stained in azocarmine, mordanted in phosphotungstic acid, and stained in aniline blue – orange G (Heidenhain–Mallory).

The parotoid glands of eight toads were completely emptied of their secretion by gentle pressure with forceps. The glands were removed, two at each time, 4½ hr., 18 hr., 72 hr., and eight days later. They were fixed in Bouin fluid and subsequently stained in hematoxylin and eosin for microscopic examination.

Results

The parotoid glands of the toad lie on the back of the head, just behind the eye and the ear, and produce a thick latex-like secretion. Each of these glands consists of a large number (180–200) of nearly spherical vesicles of varying size opening onto the surface of the gland by way of pyramidal-shape ducts (Fig. 1). The vesicles are embedded deep in the compact corium. They are surrounded by a layer of loose connective tissue containing nerves and capillaries. Within the loose connective tissue layer there is a more or less continuous layer of smooth muscle cells.

The glandular epithelium of the vesicle (Figs. 2 and 3) is made up of a continuous thin layer of cytoplasm, of varying thickness, with numerous nuclei flattened against a basement membrane. There are no discernible cell limits. The secretion of these glands is in the form of small droplets, nearly uniform in size, with which the vesicles are loaded. The droplets are formed in the cytoplasm, increase in size, and rupture into the cavity of the vesicle (Figs. 2 and 3). This seems to be a continuous process under ordinary conditions. The "warts" in the skin of the toad are identical with the parotoid glands except that they contain only three or four glandular vesicles. Mucous glands are abundant everywhere in the skin of the toad but the poison glands are found only in the skin of the dorsal surface.

The cytoplasm of the glandular vesicles stains pink with hematoxylin and eosin and the secretory droplets are strongly basophilic. Following periodate – Schiff reagent the cytoplasm is almost colorless while the droplets give a slightly positive reaction. After silver impregnation the nuclei are densely black; the cytoplasm is not blackened; and the droplets are dark gray to black. Following the Schmorl stain the cytoplasm is pale green and the droplets are

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FIG. 6. A section through the skin of a parotoid gland just above a disintegrating poison gland 72 hr. after discharging. A new poison gland is developing just under the epidermis. H. & E. × 200.

FIG. 7. A collapsed poison gland 72 hr. after discharging showing invasion of lymphocytes into the surrounding area. H. & E. × 200.

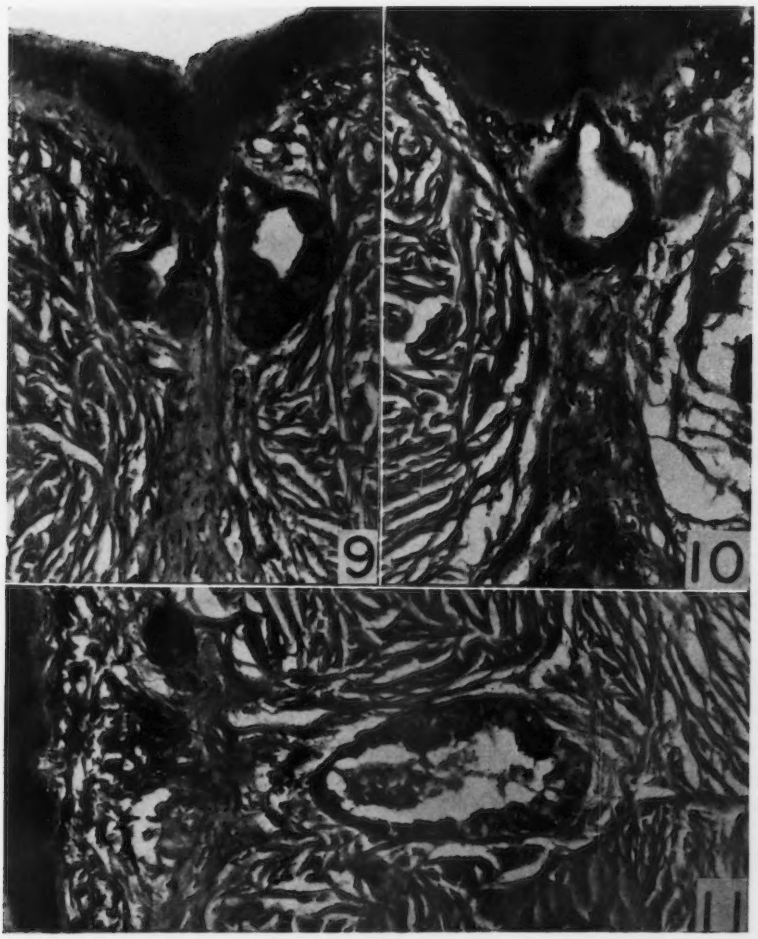
FIG. 8. A completely disintegrated poison gland eight days after discharging. H. & E. × 150.

bright green. The cytoplasm was stained pale red and the droplets purplish-red with the Heidenhain–Mallory stain. This stain also demonstrated mucin and connective tissue. The mucus of the mucous glands was also clearly revealed by the periodate – Schiff reagent and by eosin after hematoxylin and eosin.

The secretory vesicles of the parotoid glands could not be completely emptied by annoying or irritating the toads. They were, therefore, emptied completely by gentle pressure of forceps. This procedure probably resulted in some slight damage to the tissue. When examined microscopically the vesicles were seen to be entirely empty and collapsed. The space surrounding the collapsed vesicle, formerly occupied by the loaded vesicle, is loosely filled with a fibrillar extension of connective tissue (Figs. 4 and 5). Blood supply to the vesicle is thus cut off. The cytoplasm of the wall of the vesicle takes on a somewhat cellular appearance and occasional mitotic figures are seen. A great many of the formerly numerous nuclei become pyknotic and are seen at the inner edge of the cytoplasm (Figs. 4 and 5). Eighteen hours later there has begun an invasion of lymphocytes into the area surrounding the collapsed gland (Fig. 5). These have become abundant by 72 hr. (Fig. 7). The wall of the vesicle has begun to break down by 72 hr. and is completely disintegrated by eight days (Fig. 8) and ultimately disappears. Until disintegration is advanced the cytoplasm continues to form very small amounts of secretion. One or more new secretory vesicles regenerate from the Malphighian layer of the epidermis immediately above the position of the old disintegrated vesicle. These may appear as early as 72 hr. (Fig. 6) and are well established by eight days (Figs. 9 and 10) and may even have grown down into the corium to occupy the site of the former vesicle (Fig. 11). The new vesicles are cellular in the beginning but lose their cellular structure as they mature. The regenerating vesicles can be easily distinguished from the mucous glands of the skin by their structure, position, and staining reactions.

Discussion

The skin of the toad is abundantly supplied with mucous glands over the whole body. They are small, numerous, and easily identified by their staining reactions. Embedded deep in the corium of the skin on the dorsal surfaces of the body and appendages there are aggregates of two to five glandular vesicles or poison glands which constitute the warts. Larger aggregates of these poison glands, numbering about 200 and situated on the back of the head behind the eye and the ear, constitute the parotoid glands. The poison glands of *Bufo americanus* are vesicles of varying size, the glandular epithelium of which is a thin layer of acellular cytoplasm with a large number of nuclei. Under ordinary conditions secretion seems to be a continuous process. Bristol and Bartelmez (2) have described these glands in *Bufo agua* and distinguish clearly between them and the mucous glands but state that "the cells of the glandular epithelium develop to an enormous size, and when they mature they disintegrate, their entire plasm becoming the secretion."



FIGS. 9 – 11. Sections through the parotoid gland eight days after discharging the poison glands to show regenerating poison glands. H. & E. × 150.

Calmels (3) extensively described four types of glands in the skin of the toad and discussed their evolution from one type to another. From his figures and description three of these types are different phases in the secretory activity of mucous glands. The fourth type, which he called "type endothelial", is more or less identical with the poison glands described in the present paper. Calmels referred to the glandular epithelium as a flat scrap of epithelium and stated that "l'épithelium est absolument plat" but thought that it represented former poison cells. In *Bufo americanus* there are only two types of glands, the mucous glands lying close under the epidermis and easily identified by their structure and staining reactions and the deeper poison glands. The poison glands are all alike in structure but vary in size. All were functioning glands and no stages in their development were seen.

It was impossible by simply annoying or irritating the toads to cause the animals to completely discharge the contents of the poison glands. It was, therefore, necessary to empty the glands by gentle pressure. In *Bufo agua* the enormous cells of the poison glands rupture and the gland becomes merely a reservoir of poison (Bristol and Bartelmez (2)). When the poison gland is discharged it is resorbed and one of five or six undeveloped glands grouped around the neck of the functioning gland grows down to replace the remnant of the former gland. No undeveloped poison glands were seen in *Bufo americanus*. The forcibly discharged gland collapsed, and following a short period of attempted recovery in which the cytoplasm became somewhat cellular, occasional mitotic figures were seen and a small amount of secretion continued. This was only a transient condition and the poison gland disintegrated and was resorbed. The resorbed glands were replaced by new glands regenerated from the Malpighian layer of the epidermis.

All of the histochemical and histological techniques used in this study gave a positive reaction for adrenalin or adrenalin precursor substances. Abel and Macht (1) obtained two pharmacological agents from the secretion of the poison glands of the tropical toad, *Bufo agua*. One of these was adrenalin, which constituted about 7% of the secretion. The other was a substance that they called bufogen, which belongs in the digitalis series. Calmels (3) found that the secretion of an unnamed species of toad had the same effect on the frog heart as digitalis. The secretion of the poison glands of *Bufo americanus* is similar to that of the other species of toads studied.

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CONTRIBUTION À LA THÉORIE DES FEUILLETS CELLULAIRES HÉPATIQUES[1]

ROMÉO LEGAULT

Abstract

The study of both individual and serial sections of the liver of five species of fishes indicates that the parenchyma of this organ consists of a series of sheets two cells thick. These sheets are perforated, tortuous, and anastomose frequently. This description is related to Elias's conception of the vertebrate liver and shows an organization more primitive than that of mammals.

En 1949 (1), Elias rejetait la théorie classique des cordons cellulaires comme unité de base architecturale du foie des mammifères et y substituait celle des feuillets cellulaires. Il était évident qu'un travail critique du même genre s'imposait au niveau des autres classes de vertébrés. En mai 1951, une étude du foie de cinq espèces de poissons m'avait amené aux mêmes conclusions qu'Elias et Bengelsdorf (4) publiaient quelques mois plus tard dans un travail d'envergure sur le foie des vertébrés en général. Dans leur étude, les poissons sont représentés par une espèce d'Anguillidés et deux espèces de Cyprinidés. Bien que la nouvelle théorie soit maintenant présentée dans la plupart des manuels d'histologie publiés depuis, les périodiques ne contiennent aucun article corroborant la thèse d'Elias et de son groupe. Le présent communiqué tire donc sa valeur du fait qu'il est basé sur un travail fait indépendamment d'Elias et avant que celui-ci ait publié ses propres conclusions sur le sujet. Quant à l'historique de la question, on le trouvera dans Elias (2).

Matériel et méthodes

Cinq espèces de poissons ont été utilisées: la truite mouchetée (*Salvelinus f. fontinalis*), le mulet du Nord (*Semotilus a. atromaculatus*), la barbotte brune (*Ameiurus n. nebulosus*), le gougeon à fines écailles (*Chrosomos neogaeus*), et le goupie (*Lebistes reticulatus*). La méthode est celle de la reconstruction tridimensionnelle à partir de coupes épaisses de 70 à 90 microns et de coupes sériées (1).

Résultats: études tridimensionnelles

La figure 1 montre un histo-stéréogramme du foie d'*Ameiurus n. nebulosus*, obtenu en superposant les diagrammes de dix-huit coupes sériées de cinq microns chacune. Ici comme d'ailleurs pour les figures 2–5, seul le tissu parenchymateux est reproduit. Il s'agit donc d'une masse continue ayant une certaine symétrie. Au premier plan, on distingue quatre doubles rangées de cellules séparées par des espaces (lacunæ) dans lesquelles se logent le système vasculaire du foie. Vues en profondeur, ces rangées se continuent

[1]*Manuscrit reçu le neuf février 1956.*
Contribution du Département de Biologie, Université d'Ottawa, Ottawa, Ontario.

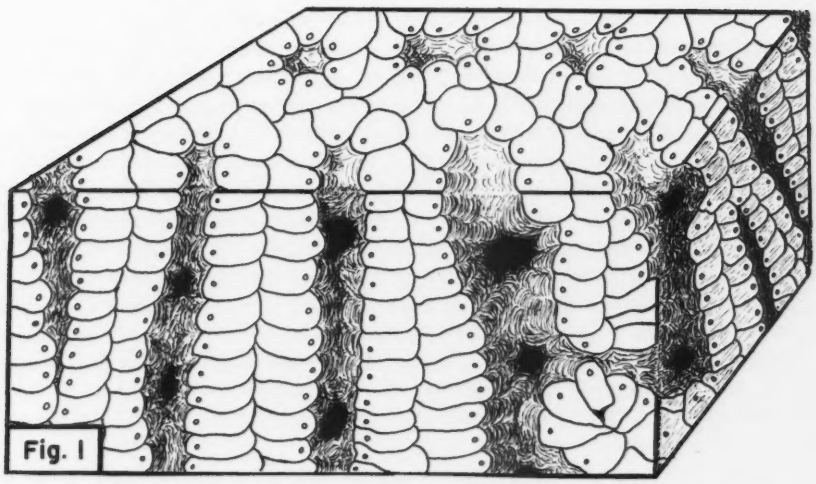

FIG. 1. Stéréogramme du parenchyme hépatique d'*Ameiurus nebulosus nebulosus*.

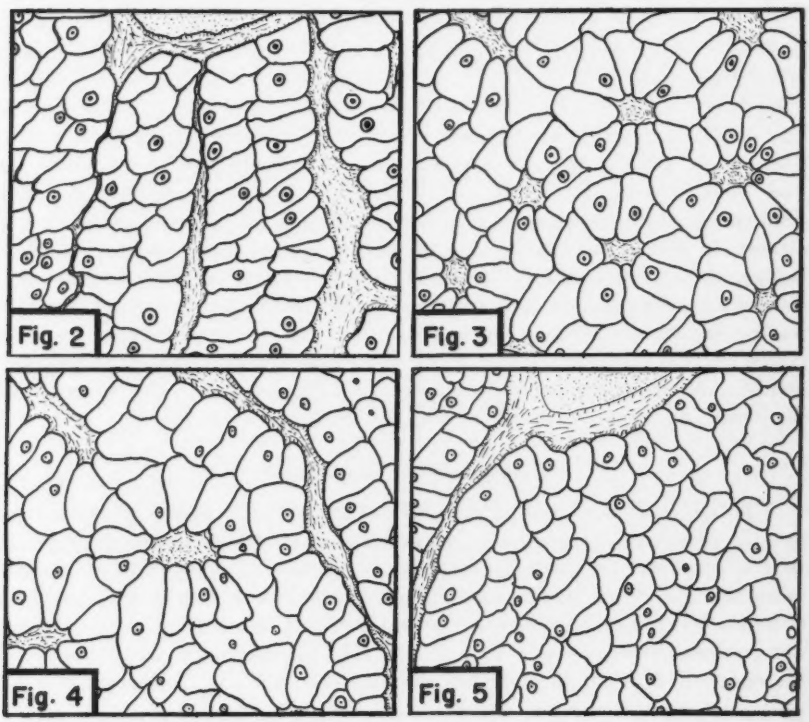

FIG. 2–5. Quatre genres de coupes histologiques du parenchyme hépatique d'*Ameiurus*.

dans le plan antéro-postérieur tout en s'anastomosant pour former d'autres lacunæ coupées transversalement. Ces doubles rangées cellulaires sont ce que nous appelerons les doubles feuillets hépatiques. Quant à la surface latérale du stéréogramme, elle offre un aspect moins régulier, ce qui s'expliquerait par la tortuosité des feuillets et de leurs anastomoses. A remarquer que ce stéréogramme, obtenu avant que nous ayions pris connaissance de celui d'Elias et Bengelsdorf (4), ressemble de très près à celui de ces derniers.

On peut donc concevoir le double feuillet hépatique comme l'élément architectural de base du foie de poisson. Ces feuillets disposés parallèlement à une distance de 20 microns ou plus, s'anastomosent fréquemment, sont tortueux et perforés, assurant ainsi la continuité du labyrinthe hépatique dans lequel le système vasculaire se loge. A l'aide de cette conception, voyons si on peut interpréter les différents genres de coupes histologiques du foie de poisson. Les figures 2–5 montrent des diagrammes de coupes de foie d'*Ameiurus* faits à la caméra lucida, et représentent les quatre principaux types de coupes des cinq espèces étudiées.

Les figures 2 et 3 ressemblent respectivement à la surface antérieure et supérieure du stéréogramme (fig. 1), et peuvent donc être interprétées de la même façon. La figure 4 serait une modification de la surface latérale de la figure 1. La tortuosité et les ajours des feuillets peuvent en effet donner toute une gamme de coupes obliques des lacunæ en plus de coupes transversales et longitudinales.

La figure 5 présente à gauche une coupe longitudinale d'une lacuna dont le mur droit se prolonge en un tissu continu. Certaines coupes de ce genre s'expliquent par le fait que l'injection n'a pas distendu tous les vaisseaux du foie. Les murs des lacunæ sont alors si rapprochés que les cellules donnent l'impression d'un tissu continu. Mais même si l'injection est parfaitement réussie, la théorie des feuillets peut expliquer ce genre de coupe. En effet, si chacune des cellules du double feuillet a un diamètre de 12 à 18 microns, le feuillet lui-même offre alors une surface de 24 à 36 microns en coupe sagittale ou parasagittale. Même si le feuillet n'est pas rigidement droit, on peut certes obtenir quelques coupes de cinq microns donnant l'aspect d'un tissu continu, au moins sur une distance de 150 à 200 microns. Ces coupes sont d'ailleurs beaucoup moins fréquentes que les autres.

Discussion et conclusion

Si le foie était constitué de cordons cellulaires, toute coupe de cet organe devrait présenter des coupes ou longitudinales ou transversales ou obliques de ces cordons. On peut théoriquement calculer la proportion statistique entre ces trois genres de coupes. En comparant ces chiffres et ceux que nous donnent les coupes histologiques, on est forcé de conclure que cette théorie des cordons cellulaires ne s'accorde pas avec la réalité. Il serait fastidieux de répéter ici le détail de cette argumentation qu'Elias (1, 2, 4) a présentée à plusieurs reprises. Que l'interprétation classique de la structure parenchymateuse du foie soit fautive, il n'y a pas lieu de s'en surprendre quand on

considère les méthodes employées; on a, en somme, tenté de décrire l'architecture d'un organe à trois dimensions en se basant sur seulement deux dimensions de coupes isolées et relativement minces.

La théorie nouvelle, que nos résultats confirment, présente cependant quelques difficultés. Il est quelquefois impossible, par exemple, de déterminer le plan du feuillet, et encore plus son étendue. Ainsi, en considérant la surface supérieure du stéréogramme (fig. 1), on pourrait concevoir les feuillets comme se dirigeant de gauche à droite au lieu d'antéro-postérieurement. Dans ce cas, ce que nous avons interprété plus haut comme étant des feuillets devient des anastomoses. Quant à leur étendue, la situation se complique en raison de l'absence de lobulation chez le poisson. On trouvera sans doute une solution à ces difficultés secondaires en appliquant la méthode tridimensionnelle au matériel embryologique.

Cette étude du développement du foie apportera également quelque lumière sur le problème de la phylogénie de cet organe qui, malgré les efforts d'Elias (2, 4), reste encore assez obscur. Ce même auteur (3) vient d'ailleurs de terminer une étude de l'embryologie du foie des vertébrés qui clarifiera peut-être la situation.

Malgré ces quelques difficultés, la théorie nouvelle semble d'accord avec les recherches sur les autres classes de vertébrés. On peut en effet distinguer trois types de foie: un premier, probablement le plus primitif, constitué uniquement de feuillets à deux cellules; un second où des feuillets d'une et de deux cellules s'entremêlent, et un dernier (celui des mammifères) composé exclusivement de feuillets à une cellule.

Résumé

L'étude de coupes histologiques isolées et sériées du foie de cinq espèces de poissons indique que le parenchyme de cet organe se résoud en une série de doubles feuillets, tortueux, ajourés et s'anastomosant fréquemment. Cette description s'apparente à celle faite récemment par Elias chez plusieurs autres espèces et démontre une organisation plus primitive que celle des mammifères.

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NUTRITIONAL STUDIES WITH PSEUDOSARCOPHAGA AFFINIS (FALL.), A DIPTEROUS PARASITE OF THE SPRUCE BUDWORM, CHORISTONEURA FUMIFERANA (CLEM.)

V. EFFECTS OF VARIOUS CONCENTRATIONS OF THE AMINO ACID MIXTURE, DEXTROSE, POTASSIUM ION, THE SALT MIXTURE, AND LARD ON GROWTH AND DEVELOPMENT; AND A SUBSTITUTE FOR LARD[1]

By H. L. House and J. S. Barlow[2]

Abstract

An improved chemically defined diet has been developed for aseptic rearing of the parasitic dipteron *Pseudosarcophaga affinis* (Fall.). The time required for 50% of the larvae to develop to the third instar was decreased from nine to five days, and the percentage of larvae that became adults was increased from 19 to 60. These improvements resulted from a reduction in the dextrose concentration to 0.5% of the diet and the inclusion of 0.4% of lard or of a mixture of fatty acids. Reducing the concentration of the salt mixture when the concentration of potassium was increased did not affect growth. The optimum concentration of amino acids was about 2.0% of the medium. Excessive concentrations of amino acids, dextrose, and the salt mixture were toxic.

Introduction

In previous papers (House and Traer (6), House (5)) methods were described for laboratory propagation of the dipterous parasite *Pseudosarcophaga affinis* (Fall.) on an artificial food. House (1–4) reported that of larvae reared aseptically on the chemically defined diet, about 84% reached the third instar, 60% became pupae, and 19% became adults; females outnumbered males by two to one. Growth of larvae was slow, 9.2 days being required for 50% of the larvae to reach the third instar, whereas comparable development is achieved on pork liver in about five days. It was observed that growth and development were accelerated by including lard in the diet.

This paper describes the effects of various concentrations of the amino acid mixture, dextrose, potassium ion, the salt mixture, and lard on growth and development of *P. affinis* reared aseptically on chemically defined diets, and describes a mixture of fatty acids that can be used to replace lard.

Materials

Pseudosarcophaga affinis (Fall.)

As in previous studies (1–4), the stock was propagated on fresh pork liver.

Food Medium

The medium used at the beginning of this investigation was identical in composition with that described previously (1), but the method of preparation was simplified. The medium was compounded in two parts. Part I was a solution containing 2.0 gm. of dextrose and the water soluble vitamins in

[1]*Manuscript received December 1, 1955.*
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20.0 ml. of water. Part II was a combination of 2.0 gm. of a mixture of amino acids, 0.75 gm. of salt mixture (No. 2, U.S.P. XII), 0.1 gm. of ribonucleic acid, 0.75 gm. of agar, 67.8 ml. of water, 10.0 ml. of an aqueous emulsion of cholesterol containing 0.1 gm. of cholesterol, and 2.2 ml. of 2 *N* sodium hydroxide. The final pH of the medium was 6.8 to 7.2.

The mixture of amino acids was prepared by grinding together a large quantity of the amino acids in the proportions used previously (1). The cholesterol emulsion was prepared by dissolving 2.5 gm. of cholesterol in 25 ml. of hot ethanol and pouring this solution into 250 ml. of hot water (near boiling) containing 0.5 ml. of polyoxyethylene sorbitan monooleate (Tween 80, Atlas Powder Co. Ltd. of Canada, Brantford, Ontario). The dry ingredients of Part II were weighed into a 250 ml. Florence flask and the water and cholesterol emulsion were added. This combination was stirred as vigorously as possible with a magnetic stirrer while the sodium hydroxide was added dropwise. Part II was autoclaved for 15 min. at 15 lb. pressure, and, when it had cooled somewhat but had not commenced to gel, 20.0 ml. of the dextrose–vitamin solution, which had been filtered through a Seitz filter, were added. About 1 ml. of the complete medium was dispensed aseptically into test tubes $\frac{3}{8}$ in. by 3 in.

In preparing media for an experiment, the concentrations of the various dietary components were calculated on the basis of 100 ml. of water in the diet. Throughout this paper, however, these concentrations are referred to as percentages of the whole diet, a reasonably close approximation.

Changes in the composition and preparation of the media that accelerated growth and development of the insect were incorporated into the diet for succeeding experiments. Additional dietary components were prepared for some experiments.

Potassium-free Salt Mixture

A salt mixture similar to the commercial preparation No. 2, U.S.P. XII, but free of potassium, was used during a part of the investigation. It consisted of 14.6 gm. of magnesium sulphate heptahydrate, 3.16 gm. of ferric citrate trihydrate, 20.19 gm. of sodium dihydrogen orthophosphate monohydrate, 28.18 gm. of monocalcium orthophosphate monohydrate, 4.15 gm. of ammonium chloride, 18.02 gm. of calcium lactate pentahydrate, and 11.70 gm. of ammonium lactate.

Fatty Acid Mixture

A substitute for lard was composed as follows: 22% palmitic acid (B.D.H. refined), 10% stearic acid (B.D.H. purified stearine), 48% oleic acid (Fisher Scientific Company, purified), 15% linoleic acid, and 5% linolenic acid (Bios Laboratories Inc.). One and one quarter grams of the fatty acid mixture and 0.625 ml. of Tween 80 per 100 ml. of water were emulsified by stirring in a Waring Blendor. During preparation of the medium, 40 ml. of the aqueous emulsion was added before the addition of the potassium hydroxide, replacing an equal volume of water.

Methods

The larvae were removed from gravid females, divided among the diets, and reared individually under aseptic conditions at a constant temperature of 23°C. and a relative humidity of 60% by methods similar to those previously reported (2). The stage of development reached by each larva was recorded daily. In evaluating the results, only larvae free of contamination were considered. Unless otherwise stated, each experiment was replicated three times, and the percentages of the larvae reaching the third instar by a given day were compared by an analysis of variance; differences significant at the 1% level were considered to be real. The ET_{50} values, or times required for 50% of the larvae to reach the third instar, were calculated by a nomographic technique (Litchfield (7)).

Preliminary tests showed that the larvae should remain on the food for at least five days after reaching the third instar. Few of the larvae removed from food on the day of ecdysis became adults, and adults that developed from larvae removed three days after ecdysis were significantly smaller than those from larvae removed from five to nine days after ecdysis ($P < .01$). At nine days after ecdysis some larvae pupated in the test tubes. Consequently, if the larvae were permitted to complete development, they were allowed to remain on the food for seven days after reaching the third instar. In the previous studies (1–4) the ET_{50} was reached on about the 10th day, and all larvae were removed from the diet on the 20th, or final day of the assay.

Various Concentrations of Dextrose and of the Amino Acid Mixture

A factorial experiment was conducted in which dextrose was supplied at 0.25, 0.5, 1.0, and 2.0% and the amino acid mixture at 0.75, 2.0, 3.0, and 4.0%. The other ingredients were supplied in the usual concentrations. Approximately 25 larvae were reared on each diet in each replicate of the experiment. The diets were evaluated by comparing the percentages of larvae in the third instar on the seventh day of the assay.

Various Concentrations of the Salt Mixture and of Potassium Ion

Some inorganic salts must be provided in the diet (4). The medium previously reported (1) contained 0.2% of a salt mixture (No. 2, U.S.P. XII), and sodium hydroxide (0.16%) was used to adjust the pH of the medium. Tests indicated that the concentration of potassium ion became limiting when the concentration of the commercial salt mixture was reduced; therefore, a potassium-free salt mixture was used in a factorial experiment to determine the optimum concentration of the salt mixture and to separate the effects of potassium from those of the salt mixture. Diets were prepared essentially as before with four levels of the potassium-free salt mixture, namely, 0.0668, 0.2, 0.4, and 0.6%, and four levels of potassium, namely, 0, 0.058, 0.115, and 0.172%, obtained by substituting potassium hydroxide for an equivalent of sodium hydroxide. In each replicate, approximately 25 larvae were reared on each diet, and the percentages of larvae reaching the third instar by the seventh day were compared.

Various Concentrations of Dextrose and of Lard

A factorial experiment was conducted in which lard was supplied in the food at 0, 0.2, 0.4, and 0.6% and dextrose at 0, 0.25, 0.5, and 2.0%. Approximately 25 larvae per diet were reared in each of four replicates, and the percentages of third instar larvae on the sixth day were compared.

A Fatty Acid Mixture Compared with Lard

A chemically defined mixture of fatty acids was used to replace lard. This mixture was supplied in the food at a level of 0.4% and the effects on growth and development were compared with those obtained with a similar quantity of lard. Forty larvae were reared on each diet in each replicate, and the percentages of larvae in the third instar on the fifth day were compared.

Results

Effects of Various Concentrations of Dextrose and of the Amino Acid Mixture

The effects of various levels of dextrose and of the amino acid mixture on larval growth are shown in Fig. 1. Dextrose fed much in excess of 0.5% of the diet had an adverse effect on larval growth. Using a lesser concentration of dextrose had little effect on growth in this experiment, though dextrose cannot be omitted entirely from the diet without injury (4). From these results the optimum level of dextrose for larval growth is regarded as about 0.5%. The amino acid mixture fulfilled the requirements for larval growth when fed at 2.0% or 3.0% of the diet (Fig. 1). Much slower growth was obtained at 0.75% and 4%; hence about 2.0% of amino acids is considered as optimum for larval growth.

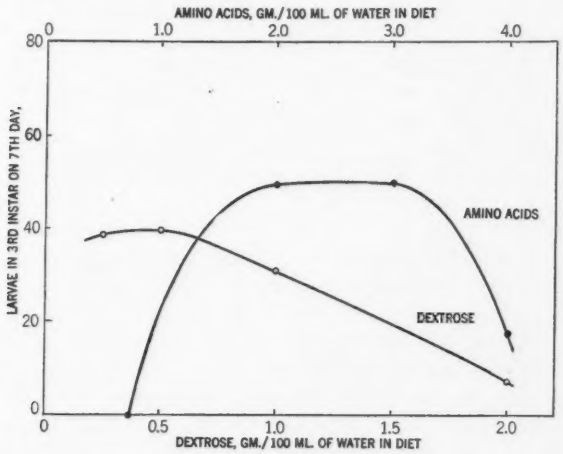

FIG. 1. The percentages of aseptic larvae that attained the third instar in seven days on various levels of dextrose and the amino acid mixture; three replicates of approximately 25 larvae each.

Analysis of variance showed that the differences in the rates of larval growth resulting from different levels of dextrose and from different levels of the amino acids were significant and that there was no indication of interaction between these components of the diet.

The median effective time required for larvae to reach the third instar (ET_{50}) on the chosen levels of dextrose and the amino acid mixture, calculated from the pooled results of the experiments by nomographic analysis, was 6.2 days with confidence limits of 6.0–6.4 days for 0.95 probability.

Effects of Various Concentrations of the Salt Mixture and of Potassium Ion

Fig. 2 shows that, by the seventh day, the percentages of third instar larvae varied with various concentrations of the salt mixture and potassium ion. The salt mixture when fed at 0.0668% and 0.2% resulted in most rapid larval growth; the greater concentrations decreased the rate of growth. Therefore, the optimum concentration of the salt mixture is regarded as approximately 0.067% of the diet. Omission of potassium ion resulted in slow growth; supplying the ion at a level of 0.058% resulted in a more rapid rate of growth, which was not affected by further increases in concentration. Hence, 0.172 gm. of potassium (i.e., 2.2 ml. of 2 *N* KOH) instead of sodium hydroxide (1) was used to adjust the pH of the medium to about neutrality.

Analysis of variance showed that the differences in the rates of growth resulting from various levels of potassium ion and of the salt mixture were significant at the 1% level and that there was no interaction between these components.

Nomographic analysis of the data pooled from the three assays of diets containing these concentrations showed that the ET_{50} for 76 larvae was 6.3 days with confidence limits of 6.1–6.5 days for 0.95 probability.

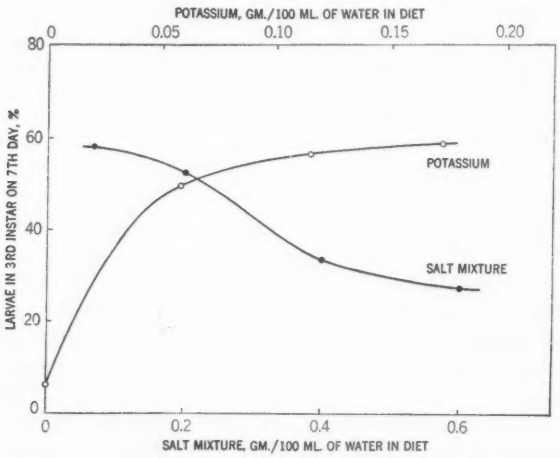

FIG. 2. The percentages of aseptic larvae that attained the third instar in seven days on various levels of the salt mixture and of potassium ion; three replicates of approximately 25 larvae each.

Effects of Various Concentrations of Dextrose and of Lard

The effects of various levels of dextrose and of lard on larval growth are shown in Fig. 3. Dextrose fed at approximately 0.5% of the diet satisfied the requirements for most rapid growth in the presence of lard; the lesser and greater concentrations resulted in slower growth.

Growth was slowed more by the omission of lard than by the omission of dextrose. Few mature larvae were obtained by the sixth day in absence of lard, whereas much greater numbers were obtained in the presence of 0.2% or more of lard. Therefore, the level of lard adopted to ensure most rapid growth was about 0.4%.

Analysis of variance showed that varying the levels of dextrose and of lard resulted in significant differences in growth rate and that there was no significant interaction between dextrose and lard.

The ET_{50} for 109 larvae reared on the diet with optimum levels of dextrose and lard was 5.6 days with confidence limits of 5.4–5.8 days for 0.95 probability.

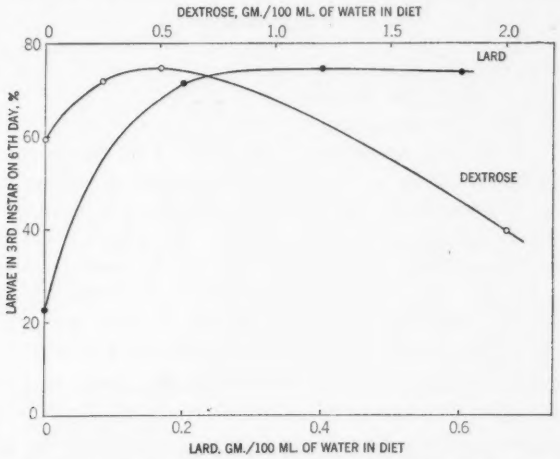

FIG. 3. The percentages of aseptic larvae that attained the third instar in six days on various levels of dextrose and of lard; four replicates of approximately 25 larvae each.

A Fatty Acid Mixture Compared with Lard

The effects of feeding lard and a mixture of fatty acids on larval growth are compared in Table I. Statistical analysis showed that the difference in the number of larvae reaching the third instar on diets containing the mixture was significantly greater than that on lard at the 5% though not at the 1% level. The (ET_{50}) on the mixture was not significantly different from that on lard ($P > .05$). Twenty-nine male and 40 female flies were obtained from 116 larvae reared with the fatty acid mixture in the diet compared with 18 male and 21 female flies from 116 larvae reared with lard in the diet. The fatty acid mixture, therefore, can be substituted for lard as a source of fats in the diet.

TABLE I

EFFECTS OF FEEDING LARD AND A MIXTURE OF FATTY ACIDS ON LARVAL GROWTH; REPLICATED THREE TIMES TO INCLUDE 116 LARVAE

Fats	Larvae in the 3rd instar by the 5th day (percentage of 116 larvae)*	ET_{50} and confidence limits for 0.95 probability (days)†
Fatty acids	81.2	4.5 (4.4 – 4.7)
Lard	58.2	5.2 (5.0 – 5.5)
Difference	23.0‡	0.7

* *By analysis of variance.*
† *By a nomographic technique.*
‡ *Significance exceeds 5% level but not 1% level.*

Discussion

These studies resulted in an improved chemically defined diet for *P. affinis*. Feeding larvae on this diet resulted in a decrease in the ET_{50} from about nine days to about five days and in an increase in the percentage of larvae that became adults from about 19 to about 60. Changes in the composition of the diet included a decrease in the concentration of dextrose and the salt mixture, and an increase in the concentration of potassium ion. Fats can now be supplied as a mixture of fatty acids. No interactions were observed between dextrose and amino acids, the salt mixture and potassium ion, and dextrose and lard in the ranges studied; hence the optimum level of these components was established by the tests. Interactions might become apparent in experiments involving suboptimum ranges of these components.

The work also confirms an earlier report (4) that dextrose promotes rapid growth of *P. affinis*, but furthermore shows that too much dextrose can be toxic, the optimum range being very narrow. Much of the cause of slow growth in earlier work (1) can be related to feeding dextrose at too high a level. On the other hand, the inclusion of the amino acid mixture at the level of 2.0% of the diet (1) has been justified, but this concentration might have been reduced somewhat had only *l*-forms of the amino acids been used.

Unpublished work by the authors had shown that growth did not occur when the diet lacked potassium and phosphate ions. This investigation showed that less salt mixture (No. 2, U.S.P. XII) is required for optimum growth than was previously reported (1) provided that the potassium ion concentration is increased. Apparently *P. affinis* larvae require more potassium and less of the other ions than is supplied by the salt mixture, which was developed to meet the requirements for certain mammals. A diet for *P. affinis* should contain about 0.17% potassium for rapid growth. The altered relationship between potassium and the salt mixture does not alter the rate

of growth. On the other hand, it is considered advantageous at this time to minimize the inclusion of substances that may affect the osmotic properties of the diet.

These studies showed that fats, in addition to cholesterol, accelerate larval growth (confirming a previous report (4)) and increase the percentage of adults obtained. Fats also tended to bring the sex ratio of the flies obtained nearer to unity; females outnumbered males by four to three. Furthermore, the requirement for fat is independent of the requirement for carbohydrate; hence the fatty acids apparently have specific roles other than calorific functions in *P. affinis*. Since a mixture of fatty acids has been developed and substituted for lard, future studies will identify the effective fatty acids and determine their specific roles.

Growth and development of *P. affinis* on the present diet are more or less comparable to its growth and development on its insect host, the prepupae of *C. fumiferana* (Clem.), and on an artificial food (5, 6); however, further work is indicated. At present there is very little larval mortality but pupal mortality is still high; fewer males than females survive to become flies. Though the flies appear to mate readily, the females only deposit eggs or chorions; no larvae have been obtained from flies reared on the chemically defined diet.

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OBSERVATIONS ON EXPERIMENTAL INFECTION OF DOGS WITH ECHINOCOCCUS[1]

By L. P. E. Choquette[2]

Abstract

Observations on the maturity of *Echinococcus granulosus* adults, their longevity *in situ*, the physical aspects of immature, mature, and senile segments eliminated in the faeces, and on the pathogenicity of the adult worm were made during the course of experimental infection of dogs.

During studies on hydatid disease attempts were made to establish experimental infection in dogs. Hydatid cysts, 3 to 10 cm. in diameter, from reindeer, elk, and moose were fed to nine young dogs free from intestinal helminths. Daily faecal examinations for the presence of eggs or segments were begun about three weeks after infection, the faeces being examined within a few hours of being passed.

There is a lack of agreement among workers as to the minimum time required after the ingestion of infective cysts by the definitive host for the eggs of *Echinococcus granulosus* to appear in the faeces. In his treatise on hydatid disease, Dévé (3) records the minimum time observed by himself and others as follows: Küchenmeister eight to nine weeks; Dévé, Leuckart, Naunyn, Railliet, Ross, seven weeks; Lorincz 36 days; van Benedin 28 days; and Siebold 27 days. According to Fiebiger (Rausch (5)), 70 days is the minimum time for the worm to mature. Figures given in textbooks vary from four to seven weeks (4, 2). Recently, Rausch (6) reported that under experimental conditions adult *E. granulosus* are present in dogs within 40 days. Rausch (5) also reported that eggs of the St. Lawrence Island form of *Echinococcus*, *Echinococcus sibiricensis* Rausch and Shiller, 1954 (7), may appear in experimentally infected arctic foxes in about four weeks.

In the present study, eight of the nine dogs fed hydatid cysts became infected, with five of them eliminating eggs and segments in their faeces. Two dogs were found to be infected 40 and 43 days, respectively, after they ate the cysts, and the other three in 51 days.

Little information is available regarding the duration of experimental infection in the dog. Krabbe (3) and Dévé (3) reported the presence of living worms in dogs killed three and one-half and four and one-half months after a single infection. Rausch (6) reported that in dogs and arctic foxes experimentally infected with the St. Lawrence form of *Echinococcus*, the worms became senile and showed a great reduction in egg production as early as three and one-half months after infection of the final host.

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Of the eight dogs infected successfully by the writer, four were kept for several months after being fed cysts. In one, fed five cysts in two lots with an interval of three weeks between each feeding, eggs and segments were recovered from the faeces for a period of eight months, after which the dog was killed and it was found to be free of worms. In another dog, fed seven cysts in two lots at a three-week interval, eggs were still being produced when the dog was killed six months after infection, and senile worms were recovered at post-mortem examination. As, in both these cases, the first signs of infection were noted 51 days after the feeding of cysts, it follows that in the first case the dog harbored immature, mature, and senile worms for nearly 10 months. In the other two dogs, fed with four and three cysts each in one dose, the first signs of infection appeared in 40 and 51 days, respectively. Post-mortem examination of these dogs, killed a week after faecal examination proved negative, failed to reveal the presence of worms.

This confirms Dévé's (3) opinion that dogs that have natural and free access to infective material—thus becoming continually reinfected—can remain contagious for very long periods of time to other animals and man.

As shown by Dévé (3) eliminated segments are easily seen on the surface of freshly passed faeces. All stools were submerged in water and examined for the presence of segments. This simple technique proved satisfactory and segments were rarely found inside the faecal mass.

Segments collected from freshly-passed faeces usually exhibited movements resulting in their shortening or elongation. When examined under the low power of the microscope, longer, translucid, and flaccid segments proved to be practically free of eggs. These were considered to be senile segments. On the other hand, translucid segments of a firmer appearance containing only a few eggs, but of the same length as segments containing numerous eggs, were considered to have discharged their eggs fairly recently. The ripe segments showed a whitish opaque spot due to accumulation of the eggs at one of the extremities (the "Eierballen" of the German workers).

Because they are embedded in the mucosa and not seen readily with the naked eye it is rather difficult to find the translucid immature worms in the intestine. Low power microscopic examination of intestinal mucosa scrapings will show the worms if present. In dead animals, the worms undergo rapid degeneration and unless post-mortem examination is performed without too great a lapse of time, the worms may not be seen with the naked eye although microscopic examination of scrapings will show evidence of infection. These observations on the appearance of the segments are similar to those made by Dévé (3) and Ross (8).

While it is agreed that dogs can harbor a great number of adult worms without apparent ill effects there are reports of pathogenicity. In his book Dévé (3) records the findings of Pillwax in 1862 and Bollinger in 1877 of haemorrhagic enteritis and rabiform symptoms in infected animals. In 1907 Dévé (3) himself noted diarrhoea, asthenia, cachexia, and death in heavily infected animals. Similarly, Brumpt in 1910 (1) noted an intense catarrhal

inflammation and death in a few days of animals that had ingested too great a number of larval parasites. More recently, Rausch (5) reported and illustrated extensive damage to the intestinal mucosa of a heavily infected dog.

Of the eight dogs infected by myself, three showed a severe diarrhoea, loss of weight, and asthenia, and death occurred within a few days of the appearance of symptoms and a month of initial infection. Post-mortem examination showed a severe haemorrhagic enteritis and a very large number of immature worms. A fourth dog began to show symptoms 43 days after the cysts were fed. The animal was killed 11 days after the symptoms appeared and examination showed a severe enteritis and several hundred mature worms.

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THREE NEW SPECIES OF SPIRURID NEMATODES FROM CANADIAN BIRDS[1]

By Patricia M. Mawson[2, 3]

Abstract

Microtetrameres canadensis from the snowy owl and the great blue heron, *Synhimantus* (*Desportesius*) *canadensis* from the bittern and great blue heron, and *Echinuria borealis* from ducks are described as new species.

Microtetrameres canadensis n. sp.

(Figs. 1 to 3)

Hosts: Snowy owl, *Nyctea scandiaca*, Cape Smith Island, Hudson's Bay. Great blue heron, *Ardea herodias*, Macdonald College, Que., and Aylmer, Ont.

Numerous females and one male are present in the collection from the owl, and one male from each of the two herons.

Female

The over-all length of the largest is 1.7 mm., the over-all width 1 mm. The body is swollen and tightly coiled (Fig. 1). In some specimens the spiral is continuous in one direction but in others it is reversed at some point; in none is the coiling haphazard. The lateral, dorsal, and ventral lines are not apparent on the outer surface and the cuticular striations are very fine. The buccal cavity has very thin walls and is of unusual shape, being cylindrical at each end with a spherical bulge in the center of its length (Fig. 2); its total length is 20 μ and that of the spherical bulge, 10 μ. The eggs are 40 by 25 μ, and the embryos are at all stages of development from morula to curled larva. The egg size agrees with that described for *Microtetrameres contorta* by Weidman (8), but the type of coiling of the worm is quite different.

Male (From the Snowy Owl)

The body is 4.4 mm. long. There are no spines on the anterior end. The head narrows suddenly, ending in six small lips around the mouth. The barrel-shaped buccal cavity is 30 μ long and 15 μ in diameter at the widest part. The anterior part of the oesophagus is 150 μ long and the posterior part 720 μ. The tapering tail is 150 μ long, and is slightly swollen at the tip. There are three pairs of preanal and four pairs of postanal papillae; these are arranged (Fig. 3) in two subventral rows with one pair of dorsolateral papillae at two-thirds of the length of the tail from the anus. The spicules are 125 μ and 3.3 mm., respectively, in length, and both end simply.

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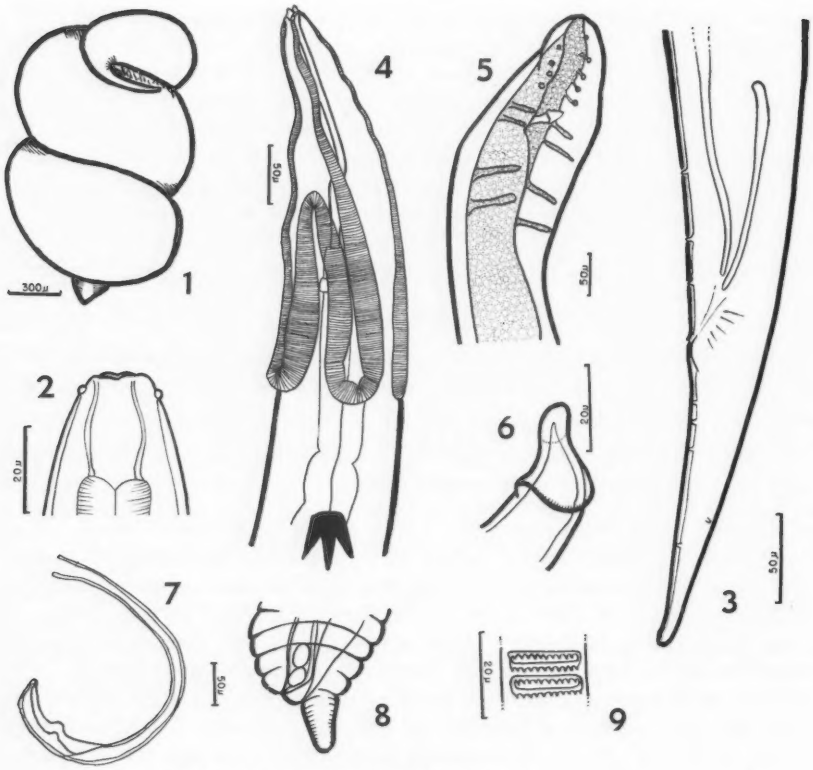


FIG. 1. *Microtetrameres canadensis*, female.
FIG. 2. *M. canadensis*, buccal cavity.
FIG. 3. *M. canadensis*, male papillae.
FIG. 4. *Synhimantus* (*Desportesius*) *canadensis*, cordons.
FIG. 5. *S.* (*D.*) *canadensis*, male caudal alae.
FIG. 6. *S.* (*D.*) *canadensis*, tip of smaller spicule.
FIG. 7. *S.* (*D.*) *canadensis*, longer spicule.
FIG. 8. *S.* (*D.*) *canadensis*, female tail.
FIG. 9. *S.* (*D.*) *canadensis*, cordons—bands of spines.

This species is closest to *Microtetrameres accipiter* Schell (7) from which it differs in the absence of flanges on the female body, in having more numerous caudal papillae, in the shape of the buccal capsule, and in the shape of the tip of the male tail.

Synhimantus (Desportesius) canadensis n. sp.

(Figs. 4 to 9)

Hosts: Bittern, *Botaurus lentiginosus* (type host), Ile Perrot, Que.
Great blue heron, *Ardea herodias*, Notre Dame du Nord, Que.

The males are 6.7 to 7.8 mm. in length, the females 7.8 to 10.9 mm. The cuticle is distinctly but not finely annulated; it is somewhat inflated into

loose rolls around the body especially at the anterior and posterior ends. The cordons (Fig. 4) are of the type with the posterior and recurrent parts of each loop wider than the anterior part which places the specimens in the subgenus *Desportesius* Chabaud and Campana-Rouget. The cordons are formed of bands of spines (Fig. 9) which are not so thickly set as those figured for *Synhimantus* (*Desportesius*) *spinulatus* by Chabaud and Campana-Rouget (3). The nerve ring surrounds the muscular oesophagus very close to its anterior end. The cervical papillae are large and trifid, the central spine being 50 μ long. In the male, the vestibule is 190 to 210 μ long, the anterior oesophagus 600 to 770 μ, and the posterior oesophagus 2.5 to 2.7 mm. long; the distance from the anterior end to the recurrent loop of cordon is 0.31 to 0.34 mm., to the anastomosis 0.14 to 0.18 mm., and to the cervical papillae 0.38 to 0.44 mm. In the female, the vestibule is 190 to 230 μ long, the anterior oesophagus 0.54 to 0.95 mm., and the posterior 2.5 to 2.7 mm.; the cervical papillae are up to 0.5 mm. and the cordons to 0.4 mm. from the anterior end.

Female

The cuticle of the tail is not folded over the posterior end in any specimen (Fig. 8), although most are completely adult with uteri crowded with eggs. The eggs are 28 to 40 μ by 18 to 20 μ.

Male

The caudal alae are very voluminous (Fig. 5). There are five pairs of postanal and four pairs of preanal papillae; in all specimens the preanal papillae are arranged on the left and right sides in two groups of two each. The spicules are distinctly unequal. The smaller forms a gutter and is about 100 μ long; the longer is cylindrical for most of its length, open and spoon-like at the distal end, and is 540 to 660 μ long (Fig. 7). The smaller spicule is spatulate, twisted at about its midlength, and its broadly rounded tip is covered by a curious thin pocket, attached as is a leather toecap to the sole of a shoe (Fig. 6).

This species resembles *Synhimantus equispiculatus* but differs from it chiefly in the size and shape of the spicules.

Echinuria borealis sp. nov.

The genus *Echinuria* Soloview is distinguished from other genera of the Acuariidae by the cordons, which are non-recurrent and anastomosing. The genus now contains at least 19 species which fall into two or four groups, according to the presence or absence of body spines and of lateral cordons, and to the relation between the cordons and the spines. These groups can be distinguished as follows:

Species with four longitudinal rows of body hooks
- Hook rows commence dorsally, anterior to anastomosis of cordons..... 1
- Hook rows commence laterally, posterior to anastomosis of cordons..... 2

Species without hooks on body
- Lateral cordons present posterior to anastomosis of true cordons..... 3
- Lateral cordons absent..... 4

Group 1 comprises the following species: *Echinuria jugadornata*, *Echinuria uncinata*, *Echinuria parva*, *Echinuria querquedulae*, *Echinuria minor*, *Echinuria hypognatha*, *Echinuria heterobrachiata*, *Echinuria borealis* (sp. nov.), and *Echinuria phoenicopteri*. These are all from Anseriformes or Charadriiformes, except the last which is from a Phoenicoptiforme bird. In all species in which it is described, the cervical papillae lie dorsal to the cordons near the level of the base of the vestibule, and in all of them they are very small.

Group 2 contains only two species: *Echinuria horrida* and *Echinuria cincta*, the first from Charadriiformes and the other from Passeriformes. In both of these species the cordons anastomose close to the head, and the rows of spines commence behind them. The cervical papillae are not described in *E. horrida;* in *E. cincta* they lie just behind the anastomosis of the cordons.

Group 3 contains the following species: *Echinuria decorata*, *Echinuria leptoptili*, *Echinuria hargilae*, *Echinuria ardeae*, *Echinuria contorta*, *Echinuria calcarata*, *Echinuria longeornata*, and *Echinuria longevaginata*. In all these species, hooks are absent from the body. In those in which the cervical papillae are described (*E. decorata*, *E. hargilae*, and *E. leptoptili*) they are situated posterior to the anastomosis of the cordons. In *E. decorata* and *E. leptoptili* the cordons are described as dentate, and in the other species they are not described in detail.

Group 4 contains a single species, *Echinuria squamata*, which differs from those of Group 3 in the presence of lateral cordons arising posterior to the cervical papillae. The cordons are all dentate and the cervical papillae, which are posterior to the cordons as in Group 3, are particularly large.

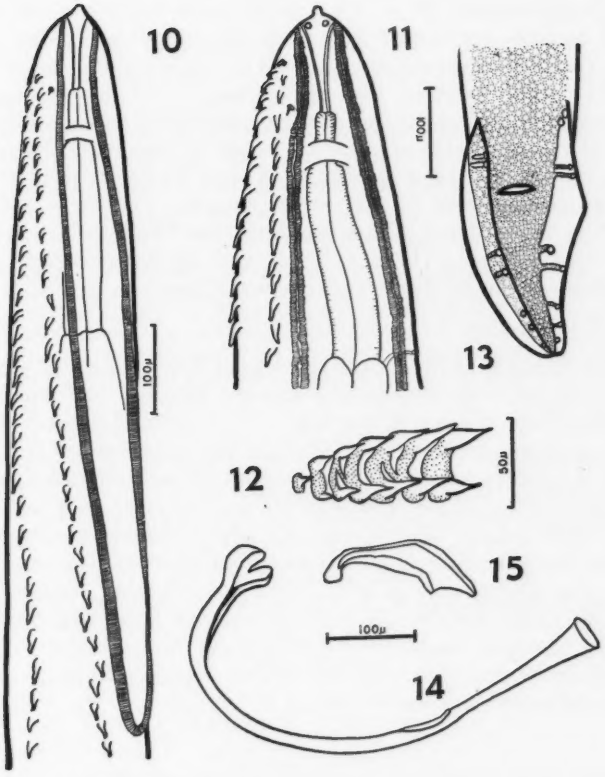
The differences listed for Group 3 above have been regarded by Alegret (1) as sufficient to necessitate the erection of a new genus *Chordocephalurus*. The differences are less striking than those which separate Groups 1, 2, and 3 from each other. Examination of the type material of the species in which there is no account of the cervical papillae or of the detailed structure of the cordons may show that they belong in reality to two or four different genera. Without seeing these specimens and the revision of the Acuariidae by Gushanskaya (5), which are not available, the present writer is unable to make more than a suggestion of the lines which such a regrouping may follow.

Echinuria borealis n. sp.

(Figs. 10 to 15)

Hosts: King eider duck, *Somateria spectabilis*, Hudson's Bay.
Old squaw duck, *Clangula hiemalis*, Hudson's Bay.

The males are 3 to 3.8 mm. and the females 3.5 to 5.3 mm. in length. The cuticle of the dorsal side is slightly swollen and has four longitudinal rows of hooks. Anteriorly, these rows of hooks are close together in pairs, so that an adjacent hook from each row arises from the same plate-like root (Fig. 12); in some cases, the roots of succeeding pairs are joined by a branch. The roots of the first 11 to 14 pairs are thus united but behind these the rows diverge



FIGS. 10 and 11. *Echinuria borealis*, cordons.
FIG. 12. *E. borealis*, hooks.
FIG. 13. *E. borealis*, papillae—male tail.
FIG. 14. *E. borealis*, longer spicule.
FIG. 15. *E. borealis*, smaller spicule.

and the hooks have separate roots. The hooks extend in males nearly to the caudal alae and in females almost to the tip of the tail. In both sexes, the size of the hooks is considerably reduced posterior to the oesophagus. The cordons of each side arise from the dorsal and ventral corners of each lip and, before anastomosing, pass back in most cases, nearly to the level of the commencement of the intestine (Figs. 10 to 11). During this traverse the cordons swing a little to the ventral side and the rows of hooks swing more to the lateral side. Each cordon is made up of two longitudinal series of rod-like cuticularizations set like roofing tiles, each one overlapping the next.

The cervical papillae have fine points; they lie between the dorsal cordon of each side and the most lateral row of hooks, at the level of the base of the vestibule.

The vestibule measures 80 to 100 μ in the male and 90 to 100 μ in the female and leads to the muscular part of the oesophagus. The muscular portion is about three times the length of the vestibule and the anterior section (60 μ) is considerably narrower than the remainder. The nerve ring and the excretory pore are at the level of the junction of the two parts of the oesophagus. The range of measurements of these structures is as follows, for male and female respectively: muscular oesophagus 0.25 to 0.3 mm., 0.27 to 0.35 mm.; glandular oesophagus 0.8 to 1.2 mm., 0.7 to 1.6 mm.; anterior end to cervical papillae 110 to 120 μ, 110 to 130 μ, to nerve ring 0.14 to 0.15 mm., 0.14 to 0.15 mm., to excretory pore 0.38 to 0.4 mm., 0.39 to 0.4 mm., and to anastomosis of cordons 0.8 to 0.9 mm., 0.79 to 1.6 mm.

Male

The cuticle and alae of the caudal regions are much inflated in some cases. There are four pairs of pedunculate postanal papillae, one small pair of sessile papillae near the tip of the tail, and four pairs of preanal papillae arranged as in Fig. 13; in some cases, as in the right side of the specimen illustrated, the two most anterior of these are absent. The longer spicule, 0.65 to 0.7 mm. in length, is cylindrical in its proximal third, then opens out to form a narrow gutter; near the tip, this gutter widens considerably and is split so that the spicule ends in two loosely approximated spoon-like pieces (Fig. 14). The smaller spicule (0.17 to 0.2 mm.) is more massive and ends in a blunt prow-like tip (Fig. 15). In two specimens the longer spicules are 0.42 and 0.47 mm. long, respectively, and in one of these specimens the shorter spicule ends in a simple tip, but no other morphological variations were observed in these two specimens.

Female

The conical tail, 0.12 to 0.15 mm. long, ends in a blunt tip. The vulva is 0.7 to 1.1 mm. from the posterior end of the body. The vagina passes back and gives rise to two long uteri. The ovaries lie one at each end of the body. The eggs are thick-shelled and embryonated, 40 to 43 μ by 25 to 27 μ.

The species is closest to *Echinuria uncinata* (4) but the cordons are longer and the male papillae are arranged differently.

Several pieces of very young specimens of *Echinuria*, as well as two whole immature worms were present in the old squaw duck. One of the worms is in process of exsheathing and the other is exsheathed. The internal proportions are similar to those described for *Echinuria borealis*. The length of the cordons, however, is much less; the hooks are a little smaller and the rows of hooks start farther back. The dorsolateral cordon makes an abrupt swing ventrad at the level of the second half of the vestibule, and at this level the dorsal rows of hooks start, while the laterodorsal start at the level of the beginning of the oesophagus, immediately behind the cervical papillae. In the exsheathing specimen this condition is seen on the outer cuticle, but the form on the new cuticle, unfortunately, could not be distinguished nor could the old cuticle be removed. No sign of sex is present.

It is considered that this condition of the hooks and cordons is probably that of a larval stage of *E. borealis*. The growth of cordons in comparison with age has been described for *Synhimantus* sp. by Chabaud (2) and for *Cosmocephalus* by Johnston and Mawson (6).

The measurements of the exsheathed worm are: length 3.25 mm., vestibule 80 μ, muscular oesophagus 0.27 mm., glandular oesophagus 1.05 mm., anterior end to the anastomosis of the cordons 0.22 mm.

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THE DEGRADATION OF HYALURONIC ACID BY MALE GONADS OF DROSOPHILA[1]

By J. A. Mutchmor and A. Glenn Richards

Abstract

The effect of extracts prepared from the reproductive systems of male *Drosophila melanogaster* on hyaluronic acid solutions has been studied; an adaptation of the viscosity reduction method was used for following the degradation of hyaluronic acid. It was found that such extracts reduce the viscosity of hyaluronic acid solutions and that the effect of the extracts is dependent on extract concentration. However, extracts prepared from whole flies have relatively little effect on the viscosity of hyaluronic acid solutions. The factor responsible for the extracts' activity is stable to heat, is dialyzable, and is not precipitated by trichloroacetic acid. The possibility that the extracts' activity is nonenzymic is discussed.

Introduction

A considerable body of information has accumulated concerning the nature of hyaluronic acid and the associated hyaluronidases, and concerning the probable importance of their relationship to certain aspects of animal physiology. Meyer (4) and Meyer and Rapport (5) have reviewed the literature and discussed the present status of knowledge regarding hyaluronic acid and hyaluronidase. Both hyaluronic acid and the enzyme are of wide occurrence; sources for the extraction of hyaluronic acid include bovine vitreous humor, pig's skin, human umbilical cord, synovial fluid, and mesenchymal tumors, while hyaluronidase is known to occur in insect and snake venoms, in various mammalian tissues including spleen, iris, and skin, associated with spermatozoa in mammalian semen, and in various microorganisms including streptococci, certain staphylococci, pneumococci, and clostridia.

Despite the wide and varied distribution of hyaluronidase, no reports have been made of attempts to determine the effects of insect reproductive system extracts on hyaluronic acid. Research undertaken to obtain such information for the males of *Drosophila melanogaster* forms the subject of the present paper.

Procedures and Materials

(*a*) *The Method*

An adaptation of the viscosity reduction method was used for following the degradation of hyaluronic acid. This method involves the measurement of changes in viscosity, with time, of hyaluronic acid solutions to which a chosen

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amount of a test extract has been added. A semimicro viscometer with an operating volume of 0.25–0.30 ml. was constructed in order to utilize extracts of fairly high concentration yet limit the time spent in dissecting fly gonads. Data, obtained as flow times, were converted to centipoise units by reference to a calibration curve constructed for the viscometer used, and values for specific viscosity, H_{sp}, were calculated. The ratio H_{sp_0}/H_{sp_x} has been used extensively in presenting the data. H_{sp_0} is the specific viscosity of the reaction mixture two minutes after initiation of the reaction, while H_{sp_x} is the value for specific viscosity at various times in the reaction period. A value of 0.880 centipoise was taken for the solvent viscosity; this figure was obtained by measuring the viscosity of a testicular extract and agrees with that calculated on the basis of molar concentrations of buffer and NaCl in the reaction mixtures.

In making the activity tests, 0.2 ml. of the substrate was pipetted into a small tube; to this was added 0.1 ml. of the extract solution. The mixture was stirred with a glass rod and drawn into the viscometer, which was immersed in a water bath at 30° C. The time at which substrate and extract were mixed was designated zero-time. The first flow time determination was made two minutes after mixing. Periodic measurements were then made on the sample during the course of the reaction until the test was terminated. The initial composition of the reaction mixture was 0.081 *M* Na_2HPO_4, 0.026 *M* citric acid, 0.7% NaCl, and 0.052% hyaluronic acid, plus various concentrations of extract.

(*b*) *The Substrate*

McIlvaine's standard buffer was prepared and diluted with one part water to two parts buffer to give a pH of 5.8, and then NaCl was added to a concentration of 0.7%. Solutions of 0.08% hyaluronic acid from umbilical cord (Nutritional Biochemical Corp.) were prepared in the saline–buffer solution shortly before each experiment and stored at 4° C. until used.

(*c*) *The Flies and Extract*

The *Drosophila melanogaster* (wild-type stock) providing the reproductive systems were reared under standard conditions on a corn meal – agar medium; gonads from four-day-old virgin males were used. The flies were anesthetized with ether and the reproductive systems removed intact through a break produced in the venter of the abdomen. The reproductive systems were accumulated in a small tube containing a known volume of the buffered saline kept ice-cold. After it was crushed with a glass rod, the homogenate was centrifuged, restirred, and extracted at 4° C. for from 8 to 12 hr. Following a second centrifugation, the supernatant was used for the activity tests. Extract concentration is expressed as the "number of reproductive systems in 0.3 ml. of buffered saline"; thus, 50/0.3 means 50 systems in 0.3 ml. of buffer solution.

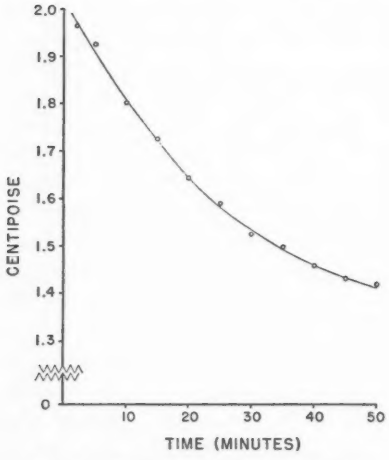

FIG. 1. The change in viscosity, with time, of a 0.08% solution of hyaluronic acid mixed with a reproductive system extract of 25/0.3 concentration.

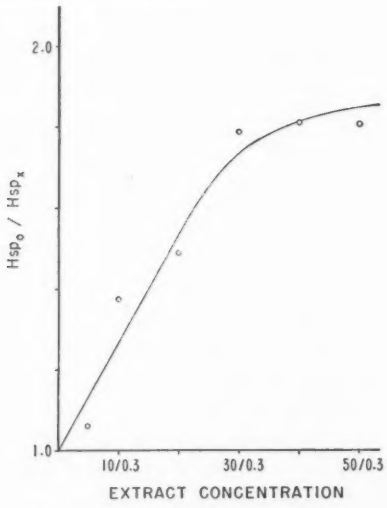

FIG. 2. The relationship of extract concentration and the extent of reduction in the viscosity of 0.08% hyaluronic acid solutions after 40 min. of reaction.

Experimental

Preliminary tests demonstrated that testicular extracts of *Drosophila melanogaster* produced marked reduction in the viscosity of hyaluronic acid solutions (Figs. 1 and 2). The effect of extract and substrate concentrations on the activity of testicular extracts has been studied. Since the interpretation of the kinetics involved is open to question the bulk of the results of these studies have not been included in this paper. However, they are included in the thesis which is on file at the University of Minnesota.

(*a*) *The Effect of Heat Treatments, Filtration, and Dialysis on the Activity of Testicular Extracts*

(*i*) *Heat Treatments*

In each test the extract to be tested was divided into two parts, one of which was subjected to a heat treatment and the other given no heat treatment prior to the activity tests. Two types of heat treatment were used; either extracts were heated in a boiling water bath for 20–30 min., or they were autoclaved for 20 min. at 240° F. and 15 lb. pressure. Extract and substrate solutions were prepared in the usual manner, and tests were begun approximately 15 min. after the heat treatment.

The effect of heating on the activity of two concentrations of extracts is summarized in Table I; the values listed are those for the ratio H_{sp_0}/H_{sp_x} at various times after initiation of the reactions. It is apparent that the heat-treated extracts have retained a large measure of their ability to degrade hyaluronic acid, although some decrease resulted from the treatment. Seven

TABLE I

THE EFFECT OF HEAT ON ACTIVITY OF TESTICULAR EXTRACTS. VALUES ARE EXPRESSED AS RATIOS OF INITIAL ACTIVITY TO THE ACTIVITY AT THE STATED TIMES (H_{sp_0}/H_{sp_x})

35 gonads per 0.3 ml.			15 gonads per 0.3 ml.		
Min.	Unheated	Heated	Min.	Unheated	Heated
2	1.000	1.000	2	1.000	1.000
10	1.298	1.047	10	0.942	1.014
20	1.678	1.153	20	1.030	1.014
40	2.380	1.455	40	1.203	1.091
60	3.200	1.855	60	1.412	1.180
90	3.870	2.385	100	1.800	1.357
145	5.220	3.175	180	2.310	1.800

such tests were made and in none of them was there a larger decrease in activity than in the tests summarized in Table I; in several tests smaller decreases occurred. The tests with water-bath heated extracts and those with autoclaved extracts gave similar results.

(*ii*) *Filtration and Trichloroacetic Acid Treatment*

An extract having a concentration of 25/0.3 was prepared and heated for 20 min. at 250° F. and 15 lb. pressure. The extract was then divided into two parts. The activity of one aliquot was tested in the usual manner to obtain a control value. The second aliquot was filtered, aided by vacuum suction, through a disk of Whatman No. 50 filter paper superimposed over a disk of fine-grade sintered glass, and a portion of the filtrate was then tested for activity (approximately one and one-half hours after heating). Crystals of trichloroacetic acid were added to the remainder of the filtrate; as the filtrate was acidified it was examined under the microscope, by reflected light, for indications of a precipitate.

For the control aliquot the values for H_{sp_0}/H_{sp_x} increased from 1.00 at two minutes after mixing to 2.02 seventy-five minutes later, whereas the values obtained for the filtered aliquot increased from 1.00 to 1.54 over the same time period. Furthermore, the addition of trichloroacetic acid to the filtrate failed to produce a precipitate. Similar results were obtained when this experiment was repeated.

(*iii*) *Dialysis*

In a preliminary experiment the activity of a testicular extract was determined before and after a 24-hr. dialysis against a comparatively large volume of buffered saline; only slight activity could be detected in the extract following dialysis. In a second experiment the extract was dialyzed against a small volume of buffered saline; activity determinations were made on the extract before and after dialysis, and on the dialyzate. For this experiment an extract having a concentration of 60/0.3 was prepared. From this 0.1 ml. of the extract was withdrawn and stored at 5° C., and 0.3 ml. was pipetted

into dialysis tubing and dialyzed against 1 ml. of buffered saline for 18 hr. at 5° C. At the end of the dialysis period 0.1 ml. was withdrawn from the solution surrounding the dialysis tube; its activity toward 0.2 ml. of 0.08% hyaluronic acid was followed viscometrically for 90 min. A similar test was made with 0.1 ml. pipetted from within the tube. Finally, the activity of the extract which had been stored at 5° C. during the dialysis period was determined; this activity is taken as representing that of the original extract.

Values for H_{sp_0}/H_{sp_x} for the extract prior to dialysis increased from 1.00 to 2.34 over a 50 min. period; activity tests, following dialysis, of the solutions within and outside the dialysis bag gave ratio increases of 1.00 to 1.20 and 1.00 to 1.11 respectively. If correction is made for dilution of the extract during dialysis, it can be calculated that the activity lost by the extract within the dialysis tube was balanced by the activity of the solution surrounding the tube.

(*b*) *Effect of 'Whole-fly' Extracts on Hyaluronic Acid*

An attempt was made to obtain comparable activity from extracts made with whole flies. An extract with a concentration of 50 flies per 0.3 ml. of extract solvent was prepared in the described manner except that whole male flies were substituted for reproductive systems. After extraction and centrifugation, 0.1 ml. of the supernatant was mixed with 0.2 ml. of 0.08% hyaluronic acid for viscosity determinations. At the end of a 90 min. test period only a slight decrease in viscosity had occurred; similar results were obtained with an extract made from flies which had been heated to 100° C. prior to extraction. Whether the extraction procedure was inadequate when whole flies were used, or some inhibition or destruction of the active factor occurs in extracts of whole flies, is not known.

(*c*) *Effect of Aging on the Activity of Testicular Extracts*

An extract having a concentration of 40/0.3 was prepared; 0.1 ml. of the extract was added to 0.2 ml. of 0.08% hyaluronic acid, and changes in viscosity were followed over a 50 min. period. The remaining extract was stored for 15 days at 4° C. and then tested for activity. In the test made prior to aging, values for H_{sp_0}/H_{sp_x} increased from 1.00 to 2.29 in 50 min., whereas after aging the values were increased from 1.00 to 1.34, indicating a decrease in activity of approximately 50%.

Discussion

Hyaluronidase appears to occupy a position intermediate between the heat-stable and the heat-labile enzymes. Emmart and Longley (1) have demonstrated that bovine testicular hyaluronidase may become spontaneously reactivated, following heat inactivation, in from 5–48 hr. Our data do not show the same phenomenon because tests were usually made as soon after heating as centrifuging and temperature equilibration permitted (about 15 min.). Our longest interval after heating was one and one-half hours, too short for a significant amount of reactivation of bovine hyaluronidase.

Numerous agents have been reported which promote the nonenzymic degradation of hyaluronic acid. The influence of metal ions on the viscosity of hyaluronic acid has been studied by Jensen (2). He found that ferrous ions (as ferrous sulphate) and cupric ions (as cupric sulphate) reduced the viscosity of the substrate while ions of zinc, mercury, cobalt, silver, lead, magnesium, beryllium, nickel, and chromium did not. The effect of ascorbic acid on the viscosity of hyaluronic acid has been studied by McClean and Hale (3). Ascorbic acid concentrations of $M/5$, $M/50$, and $M/500$ in the presence or absence of H_2O_2 produced a rapid reduction in the viscosity of hyaluronic acid. McClean and Hale report that in addition to ascorbic acid, certain reducing substances including thiolacetic acid, H_2S, quinol, pyrogallol, and sodium sulphate reduce the viscosity of the substrate. As the present study progressed it became apparent that the observed degradation of hyaluronic acid might not be due to the activity of an enzyme but might be better explained in terms of an nonenzymic process. In particular, the results of the experiments in which heat stability and dialyzability were tested give support to an nonenzymic mechanism. It is not possible to say more than this concerning the nature of the active factor. Unfortunately it is not feasible to attempt isolation of sufficient material for chemical identification because of the necessity for using extirpated gonads.

Summary

Testicular extracts of *D. melanogaster* reduce the viscosity of hyaluronic acid solutions.

The factor responsible for the extracts' activity is stable to heat, is not visibly precipitated by trichloroacetic acid, and is dialyzable.

The effect of the extracts on hyaluronic acid solutions is dependent on extract concentration.

Extract activity is reduced by aging the extract.

Extracts prepared from whole flies have relatively little effect on the viscosity of hyaluronic acid solutions.

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NOTES

SPIRURID NEMATODES FROM CANADIAN BIRDS

By Patricia M. Mawson

Among the spirurid nematodes in the collections of the Institute of Parasitology are several species which have been collected in various parts of Canada.

Habronema mansioni Seurat: this species is here recorded from the red-tailed hawk (*Buteo jamaicensis borealis*) from Moose Jaw and Swift Current, Sask., and from the duck hawk (*Falco peregrinus anatum*) from Dundas Harbour, Que.

Microtetrameres helix Cram: this parasite was found in a crow (*Corvus brachyrhynchos brachyrhynchos*) from Montreal Island, Que.

Acuaria anthuris (Rud.) (syn. *Acuaria nebraskensis* Williams): this species also was found in a crow (*Corvus brachyrhynchos brachyrhynchos*) from Montreal Island, Que.

Aviculariella alcyona Wehr: this parasite was found in a kingfisher (*Megaceryle alcyon alcyon*) from Papineau County, Que.

Echinuria uncinata (Rud.) is recorded from a mallard duck (*Anas platyrhyncos platyrhyncos*) from Alberta.

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Institute of Parasitology,
McGill University,
Macdonald College P.O., Que.

AN ADULT HEMIURUS SP. (TREMATODA) FROM SAGITTA ELEGANS VERRILL

By B. J. Myers

Six trematodes containing ova were recovered from the alimentary tract of specimens of *Sagitta elegans* Verrill, collected by Dr. A. H. Leim of the Fisheries Research Board Biological Station at St. Andrews, N.B. The trematodes, which were badly contracted, were stained in Delafield's haematoxylin. They belong to the genus *Hemiurus*. The separation of species of this genus is based on the ratio of oral to ventral sucker and on the nature of the seminal vesicle. Because neither of these could be studied accurately in this material, it is impossible to assign them to a species. They resemble most closely *Hemiurus levinseni* Odhner, 1905, in their morphology and as this is the species which is most commonly found in North American fish, it is probable that the present forms belong to it.

H. levinseni has been reported from the following fish in the North Atlantic Ocean: *Acanthocottus scropius* (Linnaeus) (8), *Clupea harengus* L. (1, 9),

Cyclopterus lumpus L. (5), *Gadus moruma* L. (2, 3, 5, 6, 7), *Lopholatius chamaelonticpes* Goode-Bean (5), *Merlucius bilinearis* (Mitchill) (5), and *Urophychis chus* (Walbaun) (6, 7).

In 1915 Cooper (1) reported this species as encysted in the muscles of small herring, while in 1927 Linton (4) described an adult hemiurid from *S. elegans* at Wood's Hole, Mass. My specimens closely resemble the latter and may be identical with it. Measurements differ somewhat but that could be accounted for by the contraction of my specimens. The oral sucker in Linton's specimens is larger than the ventral sucker, whereas the reverse is true with mine. Other differences are slight.

The presence of a sexually mature trematode in *Sagitta* is not unique but it is of interest. *Sagitta* is an abundant planktonic invertebrate with voracious feeding habits; it swallows crustacea and even young fish. *Hemiurus* is common in young plankton-feeding fish (4), and the source of infection for the fish would be the same planktonic organisms eaten by *Sagitta*. The parasite is, accordingly, probably a neotenic larva in *Sagitta*, which may serve as a source of infection for fish as a transport host rather than as an essential intermediate host.

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ANOMALOUS DEVELOPMENT OF VITELLARIA IN HEMIURUS LEVINSENI (TREMATODA)

By Leo Margolis

In the course of a survey of the parasites of North Pacific salmon (genus *Oncorhynchus*), 218 specimens of *Hemiurus levinseni* Odhner (family Hemiuridae) were collected from the pink salmon, *Oncorhynchus gorbuscha*, of which 101 specimens were encountered in a single host. From this latter lot one trematode possessed three compact vitelline glands (Fig. 1), whereas the remainder displayed two, which is the normal number in *Hemiurus*, as well as in many other Hemiuridae. Only one hemiurid genus, *Theletrum*, characteristically possesses three vitelline masses.

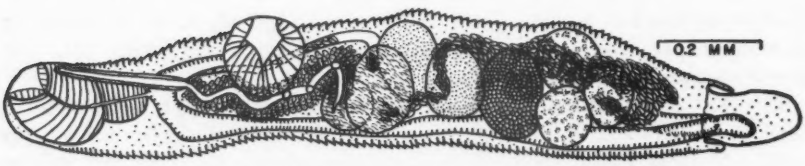

FIG. 1. Anomalous *Hemiurus levinseni* from *Oncorhynchus gorbuscha*.

To the author's knowledge, this is the first report of the occurrence of an extra vitelline mass in a trematode.

The abnormal specimen, 1.53 mm. long (including the protruding portion of the ecsoma), is considered to be a young adult because of the comparatively small number of eggs in the uterus.

The host was an adult fish returning from sea to ascend the Fraser River, British Columbia, on its spawning migration and was taken in the commercial gill-net fishery at the mouth of the river on August 1, 1955.

RECEIVED FEBRUARY 7, 1956.
FISHERIES RESEARCH BOARD OF CANADA,
PACIFIC BIOLOGICAL STATION,
NANAIMO, B.C.

HELMINTHS RECOVERED FROM DEER MICE, PEROMYSCUS MANICULATUS, TRAPPED IN THE MORGAN ARBORETUM, MACDONALD COLLEGE, PROVINCE OF QUEBEC

BY G. A. SCHAD

In the period November, 1950, to April, 1951, 57 *Peromyscus maniculatus* were trapped in the Morgan Arboretum, Ste. Anne de Bellevue, Quebec. The helminths recovered were as follows:

Parasite	Location in host	Percentage infected
Postharmostomum helicis	Caecum	7
Syphacia peromysci	Caecum and colon	84.2
Capillaria sp.	Small intestine	19.3
Mastophorus muris	Liver	1.7
Taenia tenuicollis (cyst)	Liver	3.8
Metacercaria	Liver	1.7

RECEIVED MARCH 15, 1956.
INSTITUTE OF PARASITOLOGY,
MCGILL UNIVERSITY,
MACDONALD COLLEGE P.O., QUE.

GASTROINTESTINAL PARASITES OF BEAVER IN NEWFOUNDLAND

By L. P. E. Choquette and D. H. Pimlott

The formalized gastrointestinal tracts of 89 beavers (*Castor canadensis* Kuhl), trapped during 1954 in various localities in Newfoundland were examined for the presence of parasites. Eighty of the 89 contained helminths as follows:

Travassorius americanus in 76 (85%)
Castorstrongylus castoris in 18 (20%)
Stichorchis subtriquetrus in 80 (90%)

T. americanus was present in the stomach of 70 beavers, in the small intestine of 48, in the colon of 30, and the caecum of 26. The largest number collected in any one animal was 2642; 26 beavers had over 300 worms each.

C. castoris was present in the caecum of 13 beavers, the colon of 4, and the stomach of 2. The maximum number recorded in any one animal was 14.

S. subtriquetrus was present in the caecum of 76 beavers, the colon of 17, the small intestine of 1, and the stomach of 2. Although usually present in small numbers (25 to 50), in one beaver 366 were found.

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Institute of Parasitology,
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and Natural Resources, Cormack, Nfld.

AN ADDITIONAL NOTE ON THE DISTRIBUTION OF BIACANTHA DESMODA

By Robert W. Wolfgang

Fifteen vampire bats (*Desmodus rotundus murinus*) collected from Mexico were examined for helminth parasites. These bats were loaned through the courtesy of the Museum of Natural History, Laurence, Kansas, and the author is grateful for the opportunity of examining the material. Five specimens of adult vampires contained a total of nine specimens of *Biacantha desmoda* Wolfgang, 1954. There were four females and five males. No bat contained more than three worms and one contained only a single male. In this material the cuticular expansion on the head of the female is similar to that of the male although the hooks on female worms are larger than those on males. Other than this discrepancy these specimens conform to the original description (1). *B. desmoda* was originally described from the vampire in Trinidad. The present material is from three localities: Jalpa and Zacatecas in the southeast and south central part of Mexico and San Blas on the southwest coast, thus extending the known distribution of this parasite.

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FURTHER OBSERVATIONS ON THE pH IN THE GUT AND THE BLOOD OF CANADIAN FOREST INSECTS[1]

By A. M. Heimpel[2]

The importance in insect pathology of the pH of the gut contents and the blood of insects have been discussed and determinations for a number of species of forest insects have been recorded in a previous paper (3). This contribution reports similar studies on an additional 14 species of insects.

Materials and Methods

All larvae examined had been kept in the laboratory for at least a week, and with the exception of sixth-instar larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.), which were from a group known to be infected with a microsporidian, *Perezia fumiferanae* Thom. (5), they appeared to be normal and healthy.

Special thanks are due to the Forest Insect Survey Staff of the Forest Insect Laboratory, Sault Ste. Marie, through whose efforts most of the insects were supplied.

The method employed was the same as that reported earlier (3), using a model G Beckman meter equipped with a single-drop glass electrode.

Results and Discussion

The pH readings of the gut contents and the blood of the insects examined are recorded in Table I.

Except for the species *Anisota senatoria* (A. & S.), *Datana integerrima* G. & R., and *Datana ministra* Dru., there are no reports in the literature on the pH of the gut contents and blood of the insects listed.

Grayson (2) reported the pH of the rectal contents of *A. senatoria* (range 5.5 to 6.7; mean 6.0). In the present study the hindgut and rectum were examined separately and the values obtained for the rectal contents differ from Grayson's (mean reading of hindgut 7.42, rectum 6.44); Grayson's findings for the pH of the midgut contents are confirmed (mean midgut readings: anterior, 9.0; median, 9.1; posterior, 7.8). The results obtained here are consistent with Swingle's (4) reading of pH 9.5 for the total gut contents of larvae of *D. integerrima*. Fink (1) reported a pH of 6.35 for the blood of *D. ministra*, while the mean value of 6.41 was obtained in this work.

There have been no previous reports of pH readings of the gut contents and the blood made on any of the four species of sawflies listed in Table I. However, the present findings are in general agreement with the pH readings of the other species of sawflies reported (2, 3). Under the original system of typing the distribution of pH regions in the midgut of sawflies (3), the

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TABLE I

THE pH OF THE BLOOD AND IN THE GUT OF NORMAL FEEDING LARVAE*

Species	Number examined	Instar	Blood pH	Crop pH	Midgut pH			Hind gut and rectum pH		Type
					Anterior	Median	Posterior			
Hymenoptera										
Arge sp., possibly *claviconis* (Fab.)	8	Last	7.16 7.00–7.33	—	6.75 6.46–7.40	7.62 7.20–8.22	7.59 7.23–7.92	6.52 6.24–6.80		I
Arge pectoralis (Leach)	17	Last	6.85 6.82–6.89	7.56 7.32–7.77	8.95 8.70–9.63	7.93 7.40–8.65	7.00 6.66–7.62	5.90 5.44–6.34		VII
Monoctenus fulvus (Nort.)	17	Last	6.41 6.35–6.46	6.67 6.40–7.24	7.69 7.04–8.98	8.09 7.46–8.70	7.07 6.46–7.45	5.28 4.71–5.77		II
Neodiprion swainei Midd.	14	Last	6.48 6.43–6.54		6.65 6.14–7.22	7.05 6.16–7.65	7.33 7.20–7.47	6.23 5.87–6.61		III
Lepidoptera										
Choristoneura fumiferana (Clem.)	17	Second to last	5.56 5.60–5.73	8.19 7.49–8.34	7.55 7.52–7.78	8.24 7.18–8.85	7.26 6.40–7.51	6.05 5.90–6.97		
Choristoneura fumiferana (Clem.)	13	Last	6.98 6.96–7.02	7.42 7.38–7.48	8.49 8.44–8.53	9.04 8.97–9.15	9.08 8.94–9.25	6.72 6.44–6.91		
Schizura concinna A. & S.†	17	Last	6.32 6.29–6.35	8.47 8.12–8.93	7.60 7.12–8.88	9.11 8.76–9.97	7.74 7.14–8.40	7.12 6.32–7.63		
Hyphantria textor Harr.	17	Last	6.53 6.46–6.60	9.88 9.12–10.40	8.43 7.72–8.81	8.31 7.42–8.74	7.60 6.70–8.01	6.51 6.13–6.98		
								Hind gut	Rectum	
Malacosoma pluviale (Dyar)	19	Last	6.55‡ 6.52–6.58+	8.64 8.44–8.79	9.38 9.28–9.51	9.70 9.61–9.84	8.53 8.23–9.00	6.45 6.25–6.77	5.50 5.10–5.80	
Nymphalis antiopa L.	19	Last	6.56 6.50–6.70	9.05 8.37–9.52	9.29 8.77–9.74	9.28 8.80–10.16	9.29 8.70–9.98	7.39 6.73–7.72	5.96 5.44–6.22	
Datana ministra Dru.	17	Last	6.41 6.37–6.46	8.66 8.53–8.77	9.26 8.40–9.41	9.27 8.72–9.60	9.45 8.10–9.92	5.87 5.42–6.43	4.43 4.01–4.51	
Datana integerrima G. & R.	17	Last	6.52 6.43–6.63	9.55 9.16–9.85	9.23 8.77–9.64	9.39 8.87–9.90	9.17 8.65–9.62	6.31 5.56–6.78	5.36 4.50–6.10	
Anisota senatoria (A. & S.)	24	Last	6.51 6.49–6.54	9.16 8.48–9.54	9.27 8.59–9.91	9.15 7.89–9.89	7.81 7.02–8.66	7.42 7.11–7.88	6.44 6.19–6.55	
Antherea polyphemus Cram.	6	Last	6.46 6.43–6.49	9.45 8.96–9.82	9.99 9.60–10.31	10.59 10.33–10.70	10.10 9.79–10.47	5.86 5.66–5.99	5.44 5.30–5.66	
Eacles imperialis pini Mitchener	6	Last	6.44 6.39–6.49	7.51 8.80–9.87	9.40 9.30–9.60	10.24 9.80–10.60	8.07 7.77–8.61	4.85 4.74–4.99	6.10 5.56–6.49	

* *Except for last instar spruce budworm, see text.*
† *The mean reading for the accessory salivary-gland content is pH 1.74, range 1.43–1.90.*
‡ *Mean reading + range.*

Arge pectoralis (Leach) mean midgut pH pattern is sufficiently different from any of the established six types to warrant creating a new type, Type VII, to accommodate sawfly larvae possessing this particular midgut pH distribution, *Arge clavicornis* (Leach) may be classed as having a Type I midgut pattern, *Monoctenus fulvus* (Nort.) as similar to the Type II, and *Neodiprion swainei* Midd. as having a Type III midgut.

In *Schizura concinna* A. & S., a gland attached to the common silk-gland duct was found to be filled with a clear fluid of remarkably low pH (mean 1.74). It is possible that this fluid may aid in polymerizing the silk. In this same insect, there are glands located in the abdominal wall that are capable of ejecting a very fine spray when the insect is alarmed. By placing strips of indicator paper beside the insect it was possible to show that the fluid expelled is acid and is within the range of pH 4.0 to 4.5.

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